INTRODUCTION

The first recorded description of nephrotic syndrome (NS) dates to the 15th century.[1] Hyperlipidemia has been recognized as a common finding in nephrotic patients since 1917, when hypercholesterolemia was described as a feature of nephrotic syndrome.[2] Dyslipidemia of nephrotic syndrome is known to be linked to oxidative reactions and atherosclerosis.[3] Also there is evidence for endothelial dysfunction , increased lipid oxidation and impaired activity of some antioxidant enzymes in the acute phase of idiopathic NS (INS) that, along with hyperlipidemia may increase the risk of early atherosclerosis development. Atherogenic dyslipidemia is a lipoprotein profile combining 4 specific abnormalities: borderline-high total cholesterol levels; high triglyceride (TG) concentrations; high oxidized low-density lipoprotein (LDL) particles; and low high-density lipoprotein (HDL) concentrations. It is a predisposing factor to premature coronary artery disease (CAD).[4] The lipid profile seen in NS is atherogenic.[3] Hyperlipidemia is not always connected with nephrotic disease activity and may sometimes persist for long time, especially in frequently relapsing nephrotic syndrome. Though after steroid treatment the lipid profile shows lower values than in the active state, no normalisation of indices were observed in remission.[3] Clinical trials have demonstrated that in about half of the nephrotic cases lipid profile disturbances persist during remission, which due to their clinical outcomes worsens further prognosis.[5] It is important for prognostic reasons to investigate whether patients with a history of INS have different antioxidant defense status in comparison with healthy individuals and whether this may promote early atherosclerosis.[6]

Human serum paroxonase (PON1) is tightly associated with apolipoprotein A1 in HDL and has highest activity in the liver and blood.PON1 has been implicated in the prevention of LDL lipid peroxidation and also degrades biologically active oxidized lipids in lipoprotein.[7,8] Oxidized LDL formation in the sub endothelial space of arterial wall is a key initial step in atherosclerosis. Moreover there is inverse relationship between the level of oxidized lipid products and PON1 activity. PON1 activity is reduced in human subjects who are prone to develop atherosclerosis and measurement of its activity could benefit in assessing the risk and take measures to protect individuals from atherosclerosis in future.[9,10] Nephrotic syndrome in adulthood is associated with an increased risk of coronary heart disease.[1] Myocardial Infarction in children with nephrotic syndrome has been reported but relative risk has not been calculated.[1]

Hyperlipidemia and dyslipidemia along with reduced PON1 activity raise concern for development of atherosclerosis. The pathophysiology of hyperlipidemia and dyslipidemia has not been completely understood in nephrotic syndrome. No previous study has investigated the levels PON1 activity and its relation to dyslipidemia in pediatric nephrotic syndrome and this will be the first study in Indian population.

Hence the present study was taken up to study the derangement of serum lipids and PON1 activity in nephrotic syndrome and also to know if any correlation exists between the lipid parameters and PON1 activity.

OBJECTIVES

Objectives of the present study were;

1. To find out the levels of PON1 activity in nephrotic syndrome patients.

2. To correlate PON1 activity with lipid profile

REVIEW OF LITERATURE

NEPHROTIC SYNDROME

The first recorded description of nephrotic syndrome dates to the 15th century. Today nephrotic syndrome is recognised as a common chronic illness in childhood.[1] It is 15 times more common in children than adults. The incidence is 2-3/100,000 children per year; and the majority of affected children will have steroid-sensitive minimal change disease.[1] The characteristic features of nephrotic syndrome are heavy proteinuria (>3.5 g/24 hr in adults or 40 mg/m^2/hr in children), hypoalbuminemia (<2.5 g/dL), edema and hyperlipidemia.[11] Most children (90%) with nephrotic syndrome have a form of idiopathic nephrotic syndrome, histologically minimal change nephrotic syndrome (MCNS) being the most common (90%) the others are focal segmental glomerulosclerosis (FSGS) and membranous nephropathy.[1,12] The constellation of features that characterise nephrotic syndrome develop from primary alterations of the permselectivity barrier of the glomerular capillary wall, which is no longer able to restrict the loss of protein to less than 100 mg/m^2 body surface per day.[1]

Medical complications of nephrotic syndrome are potentially serious.

They are divided into acute complications that include infections and thromboembolic disease and long term sequale of NS and its treatment on bones , growth and cardiovascular system.[1,11]

Hyperlipidemia and Dyslipidemia of nephrotic syndrome

Hyperlipidaemia with raised serum cholesterol and TG concentrations, is a hallmark of nephrotic syndrome.The classic hyperlipidemic profiles in MCNS consists of increased plasma total cholesterol, LDL-cholesterol, phospholipids, VLDL

and LDL particles.[1,12] HDL-cholesterol have been reported as high, normal or low.[13,14,15] Irrespective of changes in total HDL concentrations, its subclasses are abnormally distributed with reduction in HDL_2 and increase in HDL_3.[16] This complication results from complex interactions between disordered lipoprotein metabolism, medications and dietary factors. Increased hepatic lipoprotein synthesis in response to low plasma oncotic pressure as a consequence of the urinary loss of an as yet unidentified regulatory substance, or both is thought to play a key pathogenetic part.[10] Hyperlipidemia of nephrotic syndrome may be transient only during disease activity but other times it persists indefinitely even during remissions, more so in patients with longer and more frequent relapses.[14]

NORMAL LIPID AND LIPOPROTEIN METABOLISM [17,18]

A brief description of lipid synthesis and metabolism is presented for background.

CHOLESTEROL:

Plasma cholesterol is derived from two sources, it is synthesized by the liver and intestinal mucosa from acetyl CoA and ingested in the diet(also from bile, intestinal secretion and cells).Daily synthesis is about 1gm out of which 700 mg is endogenous and 300mg is exogenous. Half of the cholesterol is converted into bile acids (400-500mg). Trace quantities are utilized for Vit D3, steroid hormone and sex hormone synthesis, remaining is used for the formation of cell membrane. The concentration of plasma cholesterol is maintained by a balance of input and output (secretion into bile and further excretion of free biliary cholesterol and bile acids into feces. The liver is the main site for synthesis of cholesterol with conversion of acetyl CoA to mevalonic acid to cholesterol. The enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase(HMG CoA reductase) is the rate limiting enzyme in the

production of mevalonic acid, the precursor of cholesterol. Of the cholesterol formed 70-80% is quickly converted to cholesterol esters through the action of lecithin cholesterol acyl transferase(LCAT)in the plasma and transported into circulation by the lipoproteins. Cholesterol is also esterified by acyl cholesterol acyl transferase(ACAT) within the cell. The remainder is excreted into the bile and then feces. Hypercholesterolemia can result from increased synthesis, impaired output or both.Cholesterol level in blood is of primary importance due to its role in the development of atherosclerosis.

TRIGLYCERIDES (TG):

Triglycerides are the esters of fatty acids with glycerol. They constitute about 95% of tissue storage fat and are the predominant form of glyceryl esters found in plasma. TG are digested in the duodenum and proximal ileum by the action of pancreatic lipase and colipase (a protein in pancreatic secretion) in the presence of bile salts. They are hydrolyzed to glycerol, monoglycerides and fatty acids. After absorption TG are resynthesized in the intestinal epithelial cells that combines with cholesterol and apo B-48 to form chylomicrons. Chylomicrons(CM) are then secreted into the lymphatic system. At the cellular level the enzyme lipoprotein lipase (LPL) hydrolysis the TG lipoprotein complex to free fatty acids which are utilised in the body by adipose tissue, muscle cell for oxidation or stored. Hypertriglyceridemia can result from overproduction or impaired catabolism and is a component of atherogenic dyslipidemia.

LIPOPROTEINS:

Lipids synthesized in the liver and intestine are insoluble and they are transported in the plasma in macromolecular complexes called lipoproteins (Fig.

No.1). These are spherical particles with non-polar lipids (TG and cholesterol esters) in their core and more polar lipids(phospholipids and free cholesterol) oriented near the surface. They also contain one or more specific proteins called apolipoproteins that are located on their surfaces.

CLASSIFICATION OF LIPOPROTEINS

Lipoproteins have different physical and chemical properties as they contain different proportions of lipids and proteins. The characteristics and composition of various human lipoproteins are summarised in the Table.1

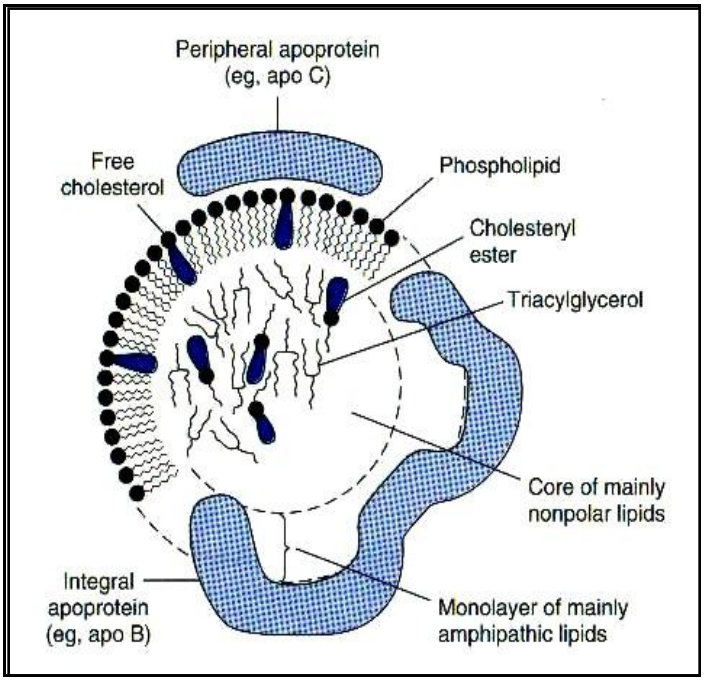


Fig No.1: Generalized structure of plasma lipoprotein.[17]

Table 1: Characteristics And Composition Of Various Human Plasma Lipoproteins.[17]

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90–1000	< 0.95	1–2	98–99	Triacylglycerol	A-I, A-II, A-IV,[1] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45–150	< 1.006	6–8	92–94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30–90	0.95–1.006	7–10	90–93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25–35	1.006–1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20–25	1.019–1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylo-microns					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[2] E
HDL_1		20–25	1.019–1.063	32	68		
HDL_2		10–20	1.063–1.125	33	67		
HDL_3		5–10	1.125–1.210	57	43		
Preβ-HDL[3]		< 5	> 1.210				A-I
Albumin/free fatty acids	Adipose tissue		> 1.281	99	1	Free fatty acids	

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
[1]Secreted with chylomicrons but transfers to HDL.
[2]Associated with HDL_2 and HDL_3 subfractions.
[3]Part of a minor fraction known as very high density lipoproteins (VHDL).

APO-LIPOPROTEINS:

The protein part of lipoprotein is called apolipoprotein (apo-Lp) or apoprotein. The physical characteristics and main functions are summarised in the Table 2. Apart from solubilising the lipid part the protein components have specific function.

(i) Modulating the activity of enzymes that act on lipoproteins

(ii) Maintaining the structural integrity of the lipoprotein complex

(iii) Facilitating the uptake of lipoprotein by acting as ligands for specific cell surface receptors.

One or more apolipoproteins are present in each lipoprotein. According to ABC nomenclature, the major apolipoprotein of HDL (α-Lipoprotein) is designated

A. The main apolipoprotein of LDL (β-lipoprotein) is apolipoprotein B and is found also in VLDL and chylomicrons. However, apoB of chylomicrons (B-48) is smaller than apo B-100 of LDL or VLDL. B-48 is synthesized in the intestine and B-100 in the liver.

Apolipoproteins C-I, C-II and C-III are smaller polypeptides freely transferable between several different lipoproteins. Carbohydrates account for approximately 5% of apoB and include mannose, galactose, fucose, glucose, glucosamine and sialic acid.

Table 2 : Apolipoproteins of human plasma lipoproteins [17]

Apolipoprotein	**Lipoprotein**	**Molecular Mass (Da)**	**Comments and Functions**
Apo A-I	HDL, chylomicrons	28,000	Reverse cholesterol transport. Activation of lecithin: cholesterol acyltransferase (LCAT). Ligand for HDL receptor.
Apo A-II	HDL, chylomicrons	17,000	Structure is two identical monomers joined by a disulfide bridge. Inhibitor of apo A-1 and LCAT?
Apo A-IV	Secreted with chylomicrons but transfers to HDL	46,000	Associated with the formation of TGl-rich lipoproteins. Function unknown. Synthesized by intestine.
Apo B-100	LDL, VLDL, IDL	550,000	VLDL secretion from liver. Ligand for LDL receptor.
Apo B-48	Chylomicrons, chylomicron remnants	260,000	Chylomicron secretion from intestine
Apo C-I	VLDL, HDL, chylomicrons	7,600	Possible activator of LCAT
Apo C-II	VLDL, HDL, chylomicrons	8,916	Activator of lipoprotein lipase
Apo C-III	VLDL, HDL, chylomicrons	8,750	Several polymorphic forms depending on content of sialic acids. Inhibits apo C-II
Apo D	Subfraction of HDL	19,300	May act as lipid transfer protein
Apo E	VLDL, IDL, HDL, chylomicrons, chylomicrons remnants	34,000	Present in excess in the β-VLDL of patients with type III hyperlipoproteinemia. The sole apoprotein found in HDL of diet induced hypercholesterolemic animals. Ligand for chylomicron remnant receptor in liver and LDL receptor.

LIPOPROTEIN METABOLISM

Can be divided into the (i) exogenous, (ii) endogenous, (iii) intracellular cholesterol transfer and (iv) reverse cholesterol transport pathways.

EXOGENOUS PATHWAY

The role of exogenous pathway is to transport dietary lipids absorbed by intestine to liver and peripheral cells which is largely mediated by CM. The pathway is summarised in the figure 2. CM are the largest of the lipoproteins and the least dense, containing 90% TG. They are synthesized in the endoplasmic reticulum of epithelial cells that line the small intestine and contain TG, other lipids with apo B-48, then move through the lymphatic system and enter the blood stream through the left subclavian vein. Here they acquire HDL additional lipoproteins apoE, and apoC's. ApoC-11 activates LPL, attached to luminal surface of endothelial cells which liberates fatty acids that are taken up by adipose tissue and muscle. As a consequence of lipolysis they are transformed to CM remnants and transfer apoA and phospholipids to HDL, the rest taken up by hepatic remnant receptor. The TG delivered to liver is oxidised to provide energy,or stored as lipid drops in cell or repackaged with apo B-100 and resecreted on VLDL particles

ENDOGENOUS PATHWAY

The primary function of the endogenous pathway is to transfer the hepatic derived lipids, especially TG to peripheral cells for energy metabolism and is summarized in Figure 3. The nascent VLDL synthesized by liver contain 55% TG VLDL along with hepatic cholesterol, apo-B-100, C's and E.

When they reach the peripheral tissues, apoC-11 activates LPL (Lipoprotein lipase) which liberates fatty acids that are taken up by adipose tissue and muscle. The remnant is now designated as IDL (intermediate density lipoprotein) and contains less of TG and more of cholesterol. This IDL contains apoB-100 and apoE. A small part of IDL is taken up by the liver, by receptor mediated endocytosis, helped by B-100 and apoE. The major fraction of IDL further loses TG and apoE so as to be converted to LDL. The TG on LDL is further depleted by cholesterol ester transfer protein (CETP) which removes TG from LDL and exchanges it for cholesteryl esters from HDL. This conversion of VLDL to IDL and then to LDL is referred to as lipoprotein cascade pathway and transfers excess surface phospholipids and apoproteins other than B-100 to HDL during this pathway. Most cells express LDL receptor but majority of LDL is returned to liver via LDL receptor which recognises apo B-100.

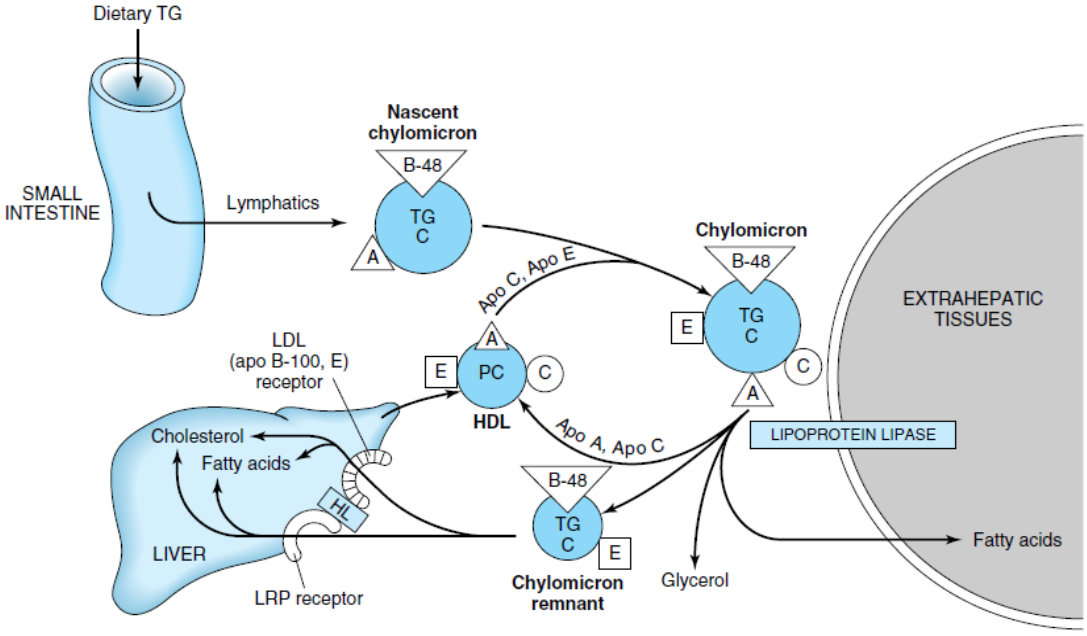


Figure 2: Exogenous lipoprotein metabolism pathway- Metabolic fate of chylomicrons.[17]

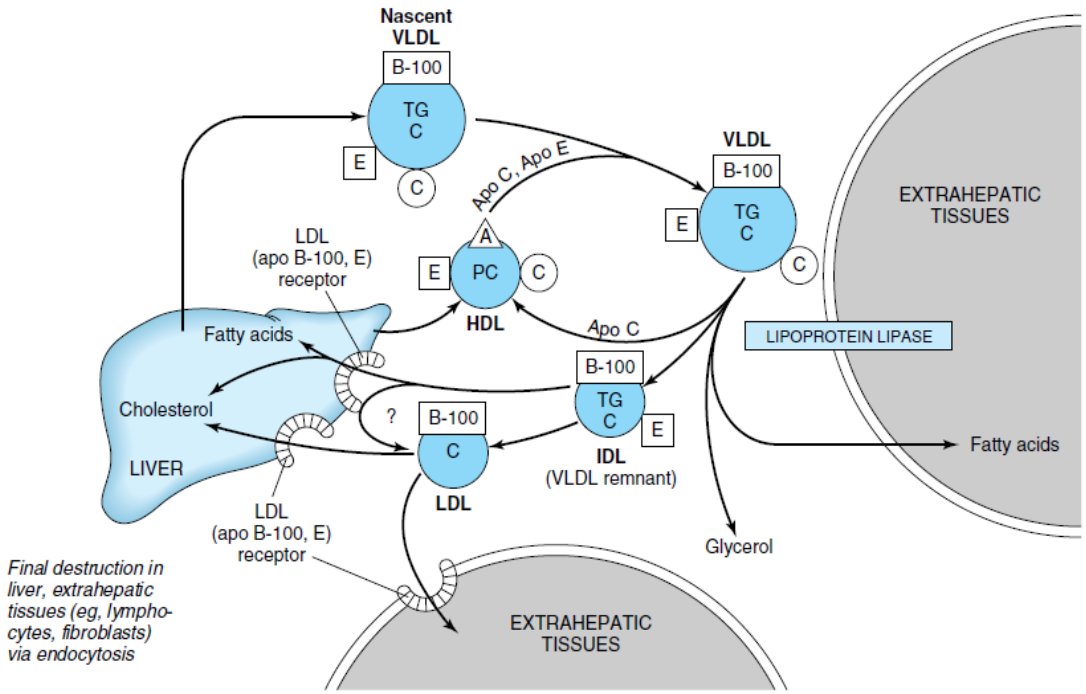


Figure 3: Endogenous lipoprotein metabolism pathway. Metabolic fate of VLDL and production of LDL. [17]

INTRACELLULAR-CHOLESTEROL TRANSPORT PATHWAY (IcCTP)

IcCTP represents the various homeostatic mechanisms that the cells use to maintain cholesterol balance and is summarized in figure 4. Excess cholesterol is toxic to the cell and it alters the biophysical properties of cell membranes. As most of the cells do not catabolize cholesterol further, any cholesterol delivered to the cell is

1. Used for membrane biogenesis
2. Steroid hormone synthesis
3. Stored in intracellular lipid drops after esterification by ACAT
4. Carried from the cell by reverse cholesterol transport pathway

Further cholesterol biosynthesis is inhibited by down regulating HMG CoA reductase enzyme and several other enzymes involved in other cholesterol biosynthesis. Excess intra-cellular cholesterol will also inhibit the expression of the LDL receptor and will also induce the synthesis of proteins involved in reverse cholesterol transport.

Hepatocytes are unique in that intracellular cholesterol has several other fates

5. Repackaged &secreted on lipoproteins
6. Converted to bile salts
7. Directly excreted in bile- only route by which cholesterol is excreted from the body

REVERSE CHOLESTEROL TRANSPORT PATHWAY (RCT)

The function of reverse cholesterol transport pathway is to remove excess cellular cholesterol from peripheral cells and return it to liver for excretion which is mediated by HDL and is summarised in figure 5. Cholesterol is pumped out of cells by ATP-binding cassette transporter-1 (ABCA1 transporter) onto lipid poor apoA-1, which binds to cells. This process results in the formation of disc-shaped nascent HDL, which is made in the liver and intestine. Discoidal HDL also interacts with ABCA1 in peripheral cells, such as macrophages and removes additional cholesterol. Lecithin-cholesterol acyl transferase (LCAT)-an enzyme, which esterifies cholesterol on HDL, plays a key role in RCT because cholesteryl esters are much more hydrophobic than cholesterol and remain trapped in the core of HDL until they are removed by the liver. The esterification of cholesterol on HDL converts the disc shaped nascent HDL to spherical HDL(HDL2). Spherical HDL (HDL2), main form of HDL in circulation also acts as extra cellular acceptor of cholesterol and acquires additional apolipoproteins, CE, and TG from HDL3. In next stage of RCT pathway liver selectively removes cholesteryl esters from lipid rich spherical HDL and lets the lipid-depleted HDL return to the circulation.

CETP also plays an important role in this pathway because a significant fraction of cholesterol that is removed from cells by HDL is transferred as cholesteryl esters onto LDL by CETP and is eventually removed from the circulation by hepatic LDL receptor. HDL2 concentrations are inversely related to the incidence of coronary atherosclerosis, possibly because they reflect the efficiency of reverse cholesterol transport.

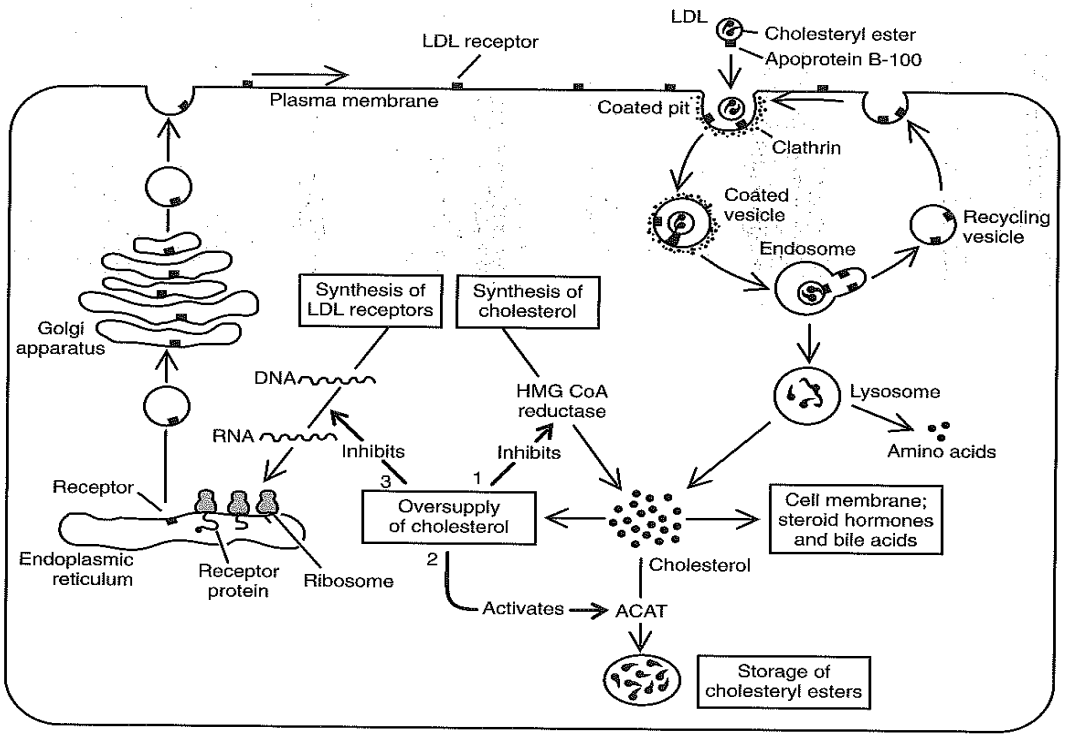


Figure 4: Intracellular-Cholesterol Transport Pathway (IcCTP) [18]

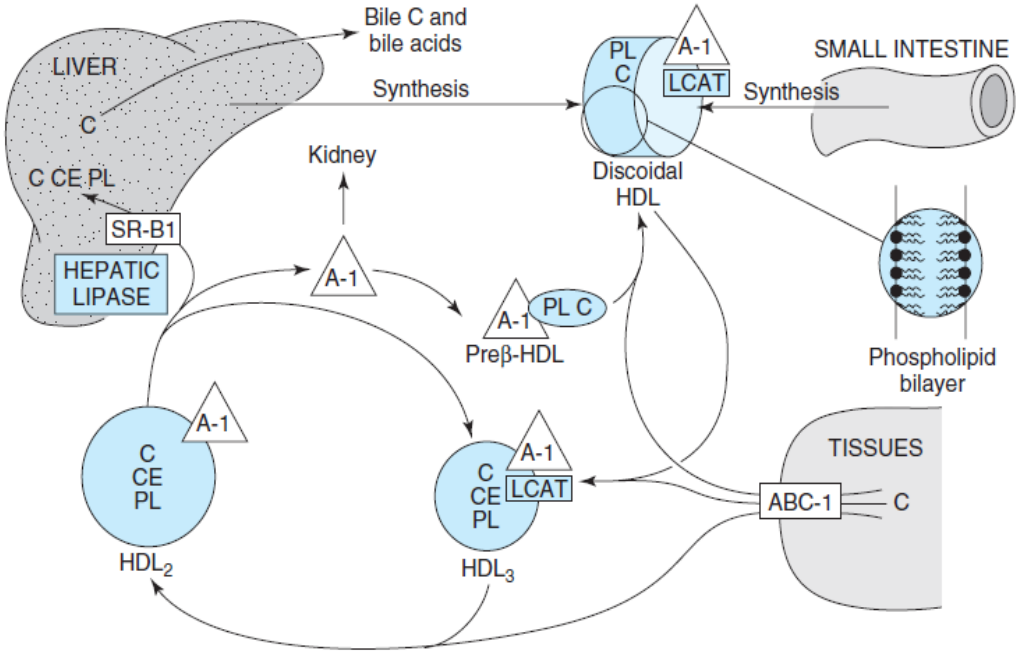


Figure 5: Metabolism of high-density lipoprotein (HDL) in reverse cholesterol transport.[17]

OXIDISED LDL (ox LDL)[18]

In presence of oxidants like superoxide and H2O2, LDL gets oxidised to form oxidised LDL which is taken up by macrophage scavenger receptors (SR-A). Unlike LDL receptors these scavenger receptors are not down regulated in response to excess intarcellular cholesterol. Hence cholesteryl esters accumulate in macrophages and cause their transformation into "foam cells" which participate in formation of atherosclerotic plaque . OxLDL has proatherogenic properties including rapid uptake by macrophages to form foam cells, chemoattraction for circulating monocytes and differentiation into macrophages and inhibition of motility of resident macrophages. It is cytotoxic and immunogenic.

LIPID AND LIPOPROTEIN METABOLISM IN NEPHROTIC SYNDROME

Hyperlipidemia with raised cholesterol and TG concentrations result from complex interactions between disordered lipoprotein metabolism, medications and dietary factors. The principle plasma lipid abnormalities in NS include increased Cholesterol, TG and Phospholipids concentrations. The magnitude of lipid abnormalities correlates with the disease severity but not with the nature of the underlying glomerular lesion.[16]

Plasma lipoprotein abnormalities include both quantitative and qualitative abnormalities.

An increase in LDL, VLDL is always seen with HDL concentrations being high, normal or low. There is a reduction in HDL_2 and increase in HDL_3 irrespective of the total HDL concentrations.[13,16] This pattern of HDL disturbance along with increased VLDL and LDL is associated with an increased risk of atherosclerosis. Further since HDL_2 is involved in recycling of apoCII to VLDL and chylomicrons

abnormal metabolism of these lipoproteins may be interrelated. Unlike primary hyperlipidemias qualitative abnormalities in composition of lipoprotein particles are present in NS. These include higher ratio of cholesterol to TG in apo-B containing particles and an increase in proportion of cholesterol, cholesterol ester and phospholipid relative to protein. [13, 16]

Apo abnormalities in nephrotic syndrome generally reflect changes in lipoprotein concentrations. Thus plasma levels of apo B, C, and E are elevated whereas apo AI and AII are no different in NS patients and controls. This is in contrast to Attman et al[13] who state marked increase in apoB , moderate increase in Apo C and E, and reduced Apo AI and AII The table summarises the various lipid, Lipoprotein and Apoprotein abnormalities in NS.

TABLE 3: Lipid, Lipoprotein and Apoprotein abnormalities in NS [16]

Lipid moiety	Increased	Decreased	Unchanged
Plasma lipids	Cholesterol TGglyceride Phospholipid		Free fatty acids
Lipoproteins	LDL, VLDL, HDL_3, Lp (a)	HDL_2	
Apoproteins	B, CII, E, CIII/CII ratio		AI, AII

Mechanisms for hyperlipidemia in nephrotic syndrome

The abnormal lipid and lipoprotein metabolism in nephrotic syndrome is summarized in figure 6.

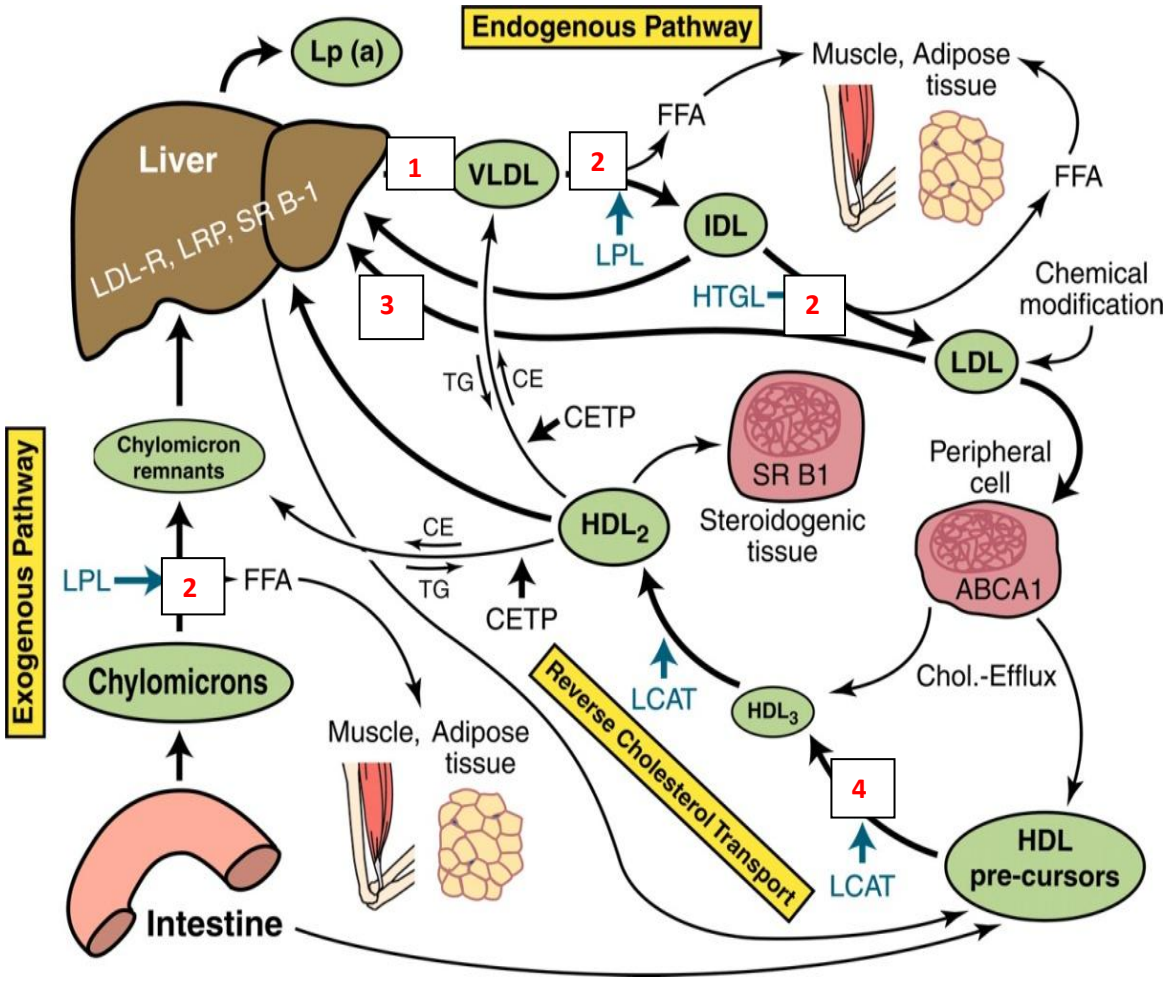


Figure 6: Normal lipoprotein metabolic pathway and proposed abnormalities in nephrotic syndrome. Refer to text for detailed explanation. Blue arrows refer to points of action of the respective enzymes in blue. Boxed numbers indicate the defects in nephrotic syndrome. 1-Increased production of hepatic VLDL leading to increased VLDL, IDL, and LDL. 2-Defective catabolism of these particles as a result of decreased LPL and hepatic Triglyceride lipase activity. 3-Impaired receptor mediated uptake of LDL. 4- Inhibition of HDL maturation as a result of diminished LCAT.

More than 30 years ago, Marsh and Drabkin postulated that in response to hypoalbuminemia in NS, hepatic production of both albumin and other plasma proteins would be increased and the synthesis of cholesterol and TG might be enhanced in parallel with that of apos.[19] But kaysen et al[20] reported that serum cholesterol concentration was completely independent of the rate of albumin synthesis. Since both albumin and dextran infusions normalised lipid and lipoprotein levels, suggests that a decrease in plasma oncotic pressure is a more important trigger for this. Proteinuria, and not an increased rate of albumin synthesis plays a causal role in nephrotic hyperlipidemia.[13,21] Furthermore, pharmacological agents that nonspecifically reduce proteinuria have shown to modify plasma lipid levels without altering the rate of hepatic protein production. These results suggest that urinary loss of a substance that regulates lipid metabolism may play a key role in pathogenesis of NS.

Turnover studies using radiolabelled glycerol and mevalonate have shown an increase in synthesis of TG and cholesterol. Increased lipoprotein production could be linked to increased supply of substrate. Since albumin is the acceptor of free fatty acids, a decreased albumin to free fatty acid ratio in NS could make more free fatty acid available for synthesis of lipoprotein.[13] Impaired renal mevalonate metabolism may lead to increased mevalonate concentrations and thus raised hepatic production of cholesterol.[12,16] This will lead to enhanced VLDL production and decreased expression of LDL receptors, thereby reducing the rate of cholesterol clearance from the circulation. Finally increased hepatic production of proteins, for example CETP are elevated which mediates transfer of esterified cholesterol from HDL to TG rich lipoproteins VLDL.

In some nephrotic patients synthesis of LDL apo B100 was actually greater than VLDL apo B100 suggesting an alternate pathway bypassing the normal delipidation pathway for increased LDL synthesis. (See Figure 3). LDL synthesis is increased but does not correlate with that of albumin. Regulation of apo B synthesis is post-transcriptional unlike the transcriptional control that regulates synthesis of most other liver derived proteins.[22]

According to Kaysen et al[22] while VLDL synthesis may be increased in some NS cases, VLDL levels increase predominantly because of decreased VLDL clearance. Measurement of LPL activity have demonstrated impaired enzyme function, this being a major cause of catabolic defect. Enzymes may be simply lost in the urine or alternatively, since albumin augments LPL by binding free fatty acids, a product of lipoprotein hydrolysis, hypoalbuminemia may result in free fatty acid accumulation which inhibits enzyme activity.[12, 16] But analbuminemia did not affect this activity. Thus urinary loss of a LPL co factor may contribute to reduced activity.[16] Candidate molecules that may influence LPL activity and can be lost in urine in NS are (i)apo CII, . Although both apo CII and apoCIII are increased CIII/CII ratio is increased. Since apo CII activates and CIII inhibits LPL such changes may contribute to defective activity of the enzyme and thus to reduced lipoprotein catabolism in NS.[14, 16] (ii) HDL particles which transfer apo CII to VLDL have been greatly lost in nephrotic urine upto 50%. Urinary losses of HDL apoAI may be 50-100% according to Short et al.[23] HDL3 is preferably lost in urine than HDL2. Normal VLDL and CM catabolism requires the presence of normally functioning HDL. The changes in subfractions of HDL reduce reverse cholesterol transport and favor atherogenesis. (iii) Glycosaminoglycans i.e. heparin sulphate which anchors LPL to

endothelium.[12, 16](iv) increased FFA:albumin ratio may act as inhibitory factor for LPL.[13]

Reduced activity of another key enzyme in lipoprotein catabolism LCAT has also been documented.[12, 16] Low LCAT impairs cholesterol esterification within the HDL particle thereby inhibiting conversion of HDL_3 to HDL_2. This in turn reduces transfer of apo CII to VLDL and thus inhibits catabolism of TG rich lipoproteins. Serum cholesterol levels were positively correlated with apolipoprotein E levels and were negatively correlated with lecithin-cholesterol acyltransferase activity in the nephrotic stage.[24]

Another abnormality that may contribute to nephrotic hyperlipidemia is defective removal of IDL and LDL from circulation via lipoprotein receptors as their expression is decreased.[16] There is increased lipid uptake by scavenger receptors in activated lymphocytes or macrophages which is unregulated.

The key metabolic abnormalities in NS are summarized in Table 4.

Table 4: Proposed mechanisms and alterations of lipoprotein metabolism for hyperlipidemia in nephrotic syndrome.[12, 13]

SYNTHETIC PATHWAY	CATABOLIC PATHWAY	RECYCLING PATHWAY
Increased availability of mevalonate	Decreased LPL activity	Decreased LCAT pathway
Decreased renal clearance of mevalonate	Decreased activators and increased inhibitors of LPL	Alterations of lipoprotein composition.
Increased FFA:albumin ratio	Impairment of VLDL clearance, decreased LDL receptors.	Decreased reverse cholesterol transport(HDL loss in urine)
Enhanced HMG-CoA reductase		
Increased cholesterol synthesis		
Increased apolipoprotein synthesis (apo B)		
Decreased oncotic pressure		

Delvin et al[12] have proposed the following scenario to explain the transient dyslipidemic component in NS. A pathogenic stimulus triggers a first line inflammatory process that leads to dysregulated cytokine production and signalling mechanisms such as IL-1, IL-18, TNFα. The raised levels of these cytokines are reported in NS by different studies. This translates into the enhanced phosphorylation of NF-kB inhibitor protein (IkBα) by IkB kinase. Studies have also shown increased levels of of NF-kB DNA binding activity and decreased levels of inhibitor protein (IkBα) in patients of NS. This will activate its transfer to the ubiquitin-proteasome for degradation and free NF-kB for its translocation to the nucleus where it will modulate the expression of a number of genes involved in lipid metabolism and in the

metabolism of cell adhesion molecules. Upon remission, the offensive stimuli taper down restoring normal kidney function and normal metabolism.

Consequences of Hyperlipidemia and Dyslipidemia of nephrotic syndrome

- The plasma lipid abnormalities seen in NS are associated with an increased risk of atherosclerosis and cardiovascular diseases in other population groups.[14, 16] With current evidences it can be stated that nephrotic syndrome is one of the causes of secondary dyslipidemia in children and they are at increased risk of atherosclerosis and cardiovascular diseases.[14,15,16,25] Hyperlipidemia, an important characteristic of nephrotic syndrome in children, is usually observed during the active phase of the disease and known to disappear with the resolution of proteinuria. However, total and LDL cholesterol levels above the 95th percentile for age and sex were also reported in 48% of nephrotic patients during remission. Hyperlipidemia induces phenotypic changes in microcirculation which are consistent with oxidative and nitrosative stresses leading to pathophysiologic features such as platelet activation, lipid peroxidation, and generation of radicals promoting atherosclerosis.[26]

- Hyperlipidemia accelerates renal injury and treatment with lipid lowering drugs may protect renal function.[14,16]

PARAOXONASE 1 (PON1) [EC 3.1.8.1]

PON1 is a 355 amino acid glycoprotein with a molecular weight of 43Kda, synthesized in the liver and secreted into the blood, where it associates with HDL. It is a member of a three gene family consisting of PON1, PON2 and PON3 located on human chromosome 7. PON1 belongs to the class of hydrolases with one of the broadest known substrate specificities.[27] Clinical interest in the serum enzyme PON1 has spanned over half a century without arriving at a consensus on its biological role. The period until the early 1990s was dominated and driven by the clinical potential of the enzyme to limit organophosphate (OP) poisoning, whether of an agricultural or military nature.[9]

In vitro assays have shown that PON1 can inhibit LDL lipid peroxidation and inactivate LDL derived oxidized phospholipids. This could potentially reduce the serum content of oxidized lipids involved in the initiation of atherosclerosis.[7] HDL from PON1 knockout mice cannot prevent oxidation of LDL in a co-culture model simulating the artery wall and their macrophages contain more oxidized lipid. As regards data from studies of human populations, these are consistent with a role for PON1 in vascular disease, without providing unambiguous proof.[28] One prospective study has shown that reduced serum PON1 activity was an independent predictor of coronary disease.[29] A wide range of other studies have shown significantly lower serum PON1 activity in patients with confirmed vascular disease or in high-risk populations.[9] Concentration and activity of PON1 are highly variable in human populations. The quantity and quality of the enzyme in serum is likely to be important in an individual's response to the risk of developing vascular disease.[9]

PON1 coding region polymorphisms [27]

There are two polymorphisms in the PON1 coding region at positions

Gln192 Arg (Q192 R) and

Leu55 Met (L55 M)

PON1 Status

In addition to genetic polymorphisms, PON1 level can be modified by acquired factors like diet, lifestyle and disease.[27] It has been shown that PON1 activities in children were very similar to values found in adults.[10]

PON1 and HDL[30]

HDL is the serum vector for PON1 and is likely to be an important determinant of enzyme concentration as the PON1 levels are reduced in HDL deficiency syndromes. PON1 tends to bind to larger sized species of HDL both in vivo and in vitro. Hence in disease like Diabetes Mellitus where HDL size is often reduced, PON1 secretion is affected. Reduced levels of serum HDL is an independent risk factor for atherosclerosis. Native HDL functions in the process of reverse cholesterol transport and also as an inhibitor of LDL oxidation. Oxidation of HDL reduces its ability to function as a potent acceptor for cholesterol efflux. HDL associated PON1 inhibits not only LDL oxidation but also HDL oxidation. This effect could be related to the ability of PON1 to hydrolyse lipoprotein associated peroxides. PON1 can hydrolyse O-P ester bond in paraoxon. A similar type of bond may exist in lipoprotein associated phospholipid peroxides and in cholesterol ester peroxides. The ability of PON1 to hydrolyse peroxides in oxidized HDL suggests that PON1 can act on already oxidized lipoprotein and reverse potential atherogenic effects. PON1

peroxidase activity is suggested since PON1 decreased cholesterol linoleate hydroperoxide levels in oxidized HDL and produced cholesteryl linoleate hydroxides.

Mechanism of PON1 binding to HDL[27]

PON1 is unusual in retaining its N-terminal hydrophobic signal sequence upon secretion from the cell. An N-terminal PON1 mutant, in which the signal sequence is removed before secretion of the protein was unable to bind to HDL. This shows that PON1 binds to HDL via its hydrophobic N-terminal signal sequence. A secondary structure prediction of the N-terminal signal sequence shows that the entire region is compatible with a trans-membrane helix. The majority of the N-terminal is disordered and invisible in the tertiary structure, but its hydrophilic part forms a helix. The hydrophobic residues of a second helix, adjacent to the N-terminal helix are oriented towards the solvent as are a number of hydrophobic residues in the loops that connect the helix to the main PON1 structure. These regions provide adjacent hydrophobic areas which would allow PON1 to bind to membranes or lipoproteins. The potential interface with HDL has an "aromatic belt" rich in tryptophan and tyrosine residues which has been described in a number of membrane binding proteins.

PON1 1 secretion and association with cell membranes

PON1 is synthesized in liver and secreted into serum.

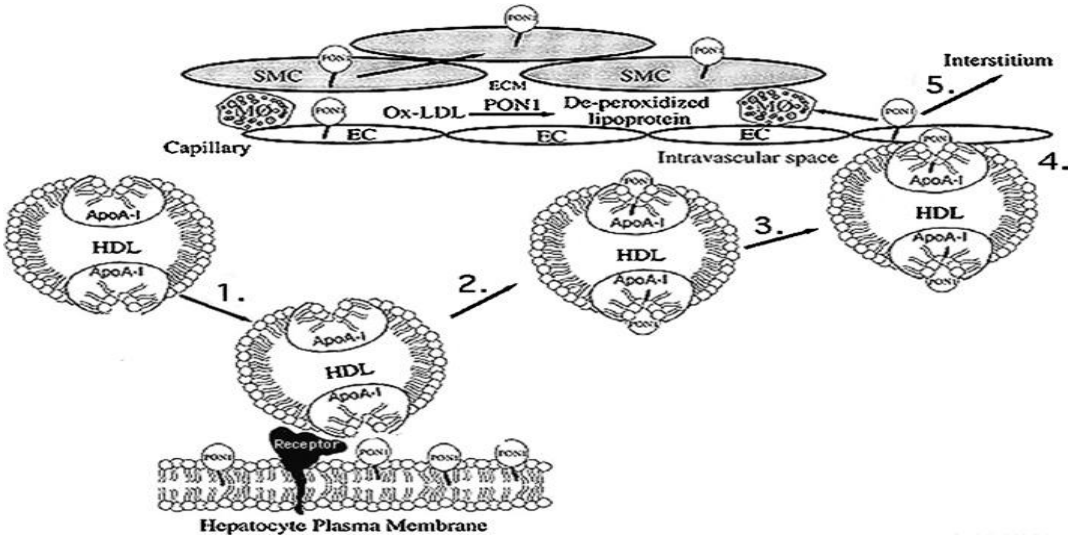


Figure 7: Proposed model for PON1 secretion, association with HDL and transfer to sites of lipid damage[27]

(1) HDL is transiently bound to the cell surface via a receptor.

(2) PON1 anchored in the cell membrane via its hydrophobic N-terminus is transferred to HDL under non-equilibrium conditions where it is stabilised by apoAI.

(3) PON1 enters the intravascular space with HDL.

(4) Under more static conditions favouring diffusion, PON1 could transfer to phospholipids in plasma membranes, possibly during receptor mediated recruitment of cholesterol from endothelial or smooth muscle cells.

(5) PON1 may therefore have access to the interstitium and areas of LDL accumulation and oxidative damage where it could protect against adverse effects of oxidation.

The retained hydrophobic N-terminal signal peptide is represented by a thick black line.

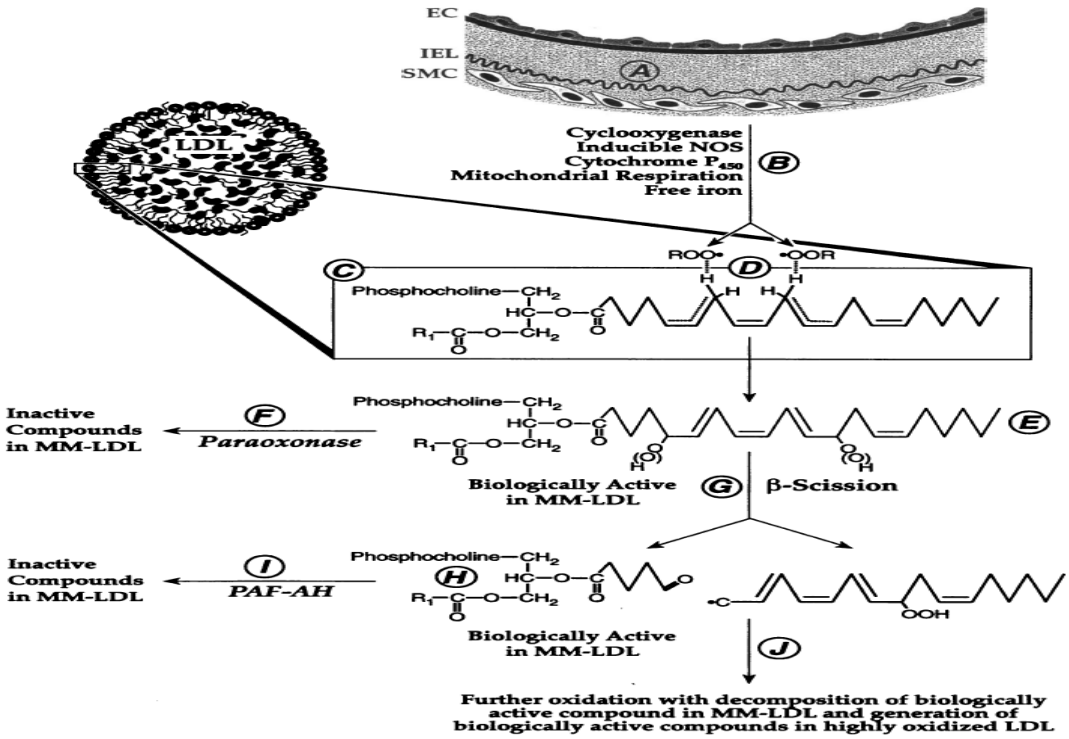


Figure 8: Hypothetical model for the mechanism by which HDL destroys biologically active lipids in mildly oxidized LDL (MM-LDL).[31] Reactive oxygen species may be formed in the artery wall (A) in areas sequestered from plasma antioxidants by a variety of possible mechanisms, some of which may involve cyclooxygenase, lipoxygenase, inducible nitri oxide synthase, cytochrome P_{450}, mitochondrial respiration, or free iron (B). These oxygen radicals may then seed LDL within the subendothelial space by oxidation of phospholipids in LDL (C). This process involves abstraction of hydrogen atoms from bis-allylic methylenes (D). Addition of molecular oxygen at multiple sites may generate multioxygenated phospholipids (E), which may be substrates for PON1 in HDL (F). In cases in which PON1 concentrations are low or depleted or in which lipid peroxide levels are excessive, oxidized phospholipids may undergo oxidative fragmentation (G) to form molecules (H) that evoke the characteristic inflammatory responses in endothelial cells induced by MM-LDL. These oxidatively fragmented phospholipids may then be substrates for the second line of defense, PAF acetylhydrolase (PAF-AH) (I). PAF-AH hydrolyzes these biologically active lipids into molecules that do not evoke the characteristic inflammatory responses in endothelial cells induced by MM-LDL. Further oxidative decomposition of lipids in LDL, including cholesteryl esters, leads to the deposition of highly oxidized LDL (J) present in the necrotic core of advanced atherosclerotic lesions.

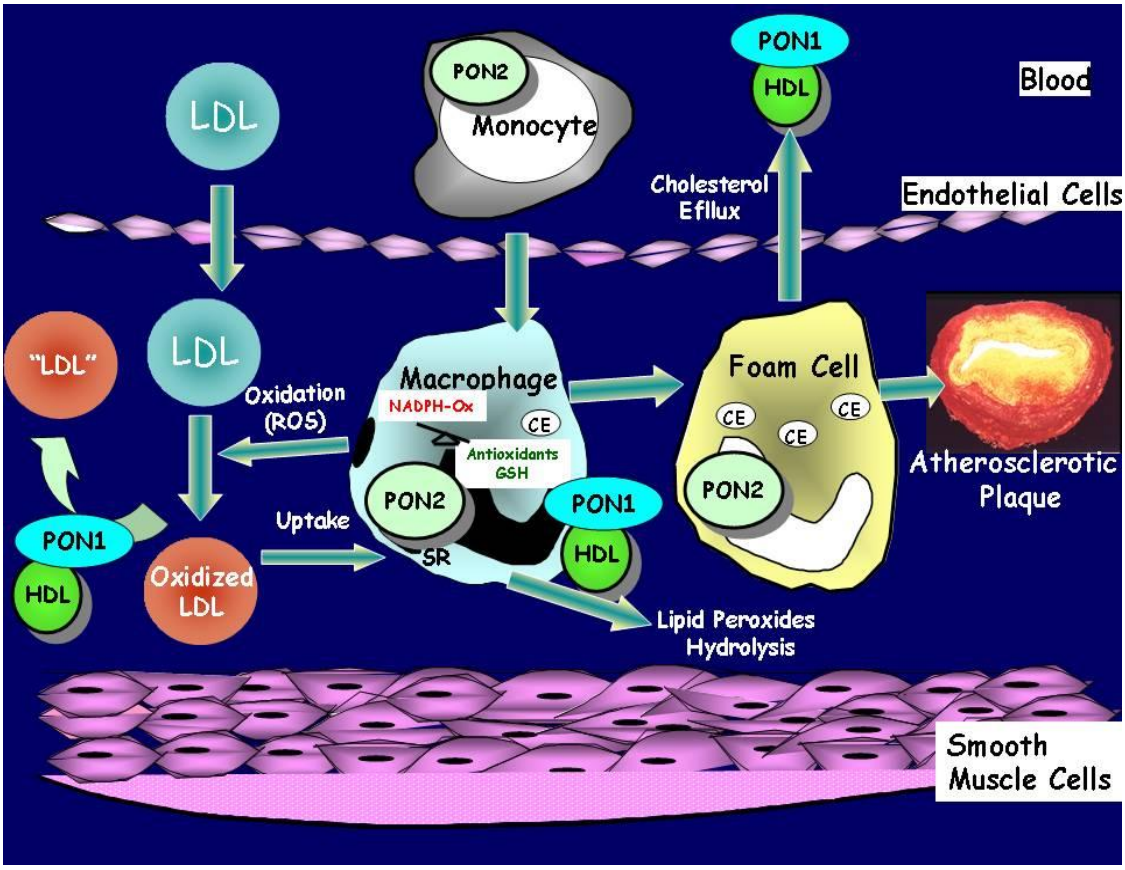


Figure – 9 Mechanism of action of PON1- Protective role of PON1 against oxidized LDL

Pharmacological Regulation of PON1[27]

Clinical studies in patients on Fibrate treatment in whom PON1 concentration and activity were measured have given conflicting results. And same results were obtained on patients with Statin therapy. Serum activity of the enzyme may also be amenable to pharmacological manipulation. Statins, the drug class most widely used to lower serum LDL, are associated with improved serum concentrations and activities of PON1.

Estimation of PON1:

The structural requirements for PON1's arylesterase and PON1 activities are not the same as those required for its protective effect against LDL oxidation and

therefore may contribute in different ways, perhaps synergistically to reduce LDL oxidation and possibly impede atherogenesis.[32] The hypothesised ability of PON1 to prevent or limit oxidation prompted attempts to identify potential substrates in the form of oxidised lipids. Several candidates have been proposed (Cholesterol linoleate hydroperoxide, Oxidised 1-palmitoyl-2-arachidonoyl-sn-, glycero-3-phosphorylcho line (Ox-PAPC), Oxidised linoleic acid). For a number of reasons like difficulty in identifying and analysing the oxidised lipids there has been little progress in identifying oxidised lipids as substrates for PON1.[9] In the continued absence of a defined physiological substrate for PON1, paraoxon (PON1 activity) and phenylacetate (arylesterase activity) remain the principal means of monitoring enzyme activity .

p-nitrophenylacetate can be used instead of paraoxon since even the former on hydrolysis gives p-nitrophenol and can be measured. In a study by Dantoine TF et al[33] they used three substrates for measuring PON1 activity viz paraoxon , phenyl acetate and p-nitrophenyl acetate. They found that p-nitrophenyl acetate to be very sensitive and specific in detecting variations in PON1 activity .It provides higher activities within narrower ranges and diminishes analytical errors. Moreover p-nitrophenyl acetate is a nontoxic ester unlike paraoxon and thus is more suitable for routine determinations.[33]

LIPID PROFILE IN NEPHROTIC SYNDROME

Baxter et al[34] in 1960 obtained sera periodically from 44 hospitalized patients with uncomplicated nephrosis (nephrotic syndrome) and found elevated levels of cholesterol , phospholipids, TG and various lipoproteins. They also reported decreased levels of albumin. Concentrations of total serum cholesterol, phospholipid,

triglyceride, and total lipid were related inversely and nonlinearly to serum albumin levels.

Mallik, Stone MC and Chopra[35] appreciated that, in both children and adults with nephrotic syndrome, the concentration of VLDL cholesterol and LDL cholesterol are often increased markedly and HDL cholesterol is usually normal or elevated in children, although the ratio of HDL cholesterol to total cholesterol may still be low. Some children with lesions other than minimal change may have decreased HDL cholesterol.

Gheradi E Rota and Calandra[36] studied serum lipoproteins in patients with untreated, uncomplicated nephrotic syndrome, and found that they had elevated VLDL, LDL, IDL and diminished HDL concentration. All the changes appear to be strictly correlated in the concentration of serum albumin and were most pronounced in those patients with serum albumin concentration less than 2.0 gm%.

Appel G.B, Blum and Chien[37] found out that, children with primary nephrotic syndrome develop hypercholesterolemia and hyperlipidemia, the increase being most marked in patients with minimal change disease. In general there is inverse correlation between concentration of serum albumin and that of cholesterol.

Querfeld U[38] investigated lipoprotein profiles in 24 children with normal renal function at different stages of the idiopathic NS. All patients with active NS had significantly elevated TC levels. TG and cholesterol in the VLDL, IDL, LDL, and cholesterol in HDL_2, but not HDL_3 were inversely correlated with the serum albumin level.

PON1 IN HEALTHY AND IN NEPHROTIC SYNDROME

Ece et al[39] showed that patients in the active phase of NS had significantly lower PON and total antioxidant response(TAR) levels and higher oxidative stress index(OSI) and total peroxide values than those in full remission. Significant correlations were found between PON, TAR and total peroxide. Serum total protein had a significantly positive correlation with PON and negative correlation with total peroxide in acute-period NS patients. Low-dose alternate-day steroids do not seem to decrease oxidative stress even when proteinuria ceases. Despite some conflicting data increased oxidation and/or decreased antioxidant response may be related to the pathogenesis of steroid-sensitive NS.

Kniażewska M H et al[6] aimed to evaluate constituents of antioxidant defense (total antioxidant potential : ferric-reducing antioxidant power (FRAP), PON1, tocopherols, ascorbic acid] in patients formerly treated for INS. The studied group consisted of 30 patients treated 4-15 years ago for INS. The control group consisted of 30 healthy teenagers. There were no statistically significant differences in PON-1 activity, alpha-tocopherol levels and sum of beta- and gamma-tocopherols, and in FRAP between groups. The results of this study suggest that the nonenzymatic antioxidant defense in young persons formerly treated for INS is weaker than in their healthy counterparts.

CORRELATION OF PON1 WITH LIPID PARAMETERS.

Saha N et al[40] studied the relation between PON and lipoproteins . In males, there was a significant negative correlation of serum PON1 activity with total and LDL cholesterol levels, and positive correlation with HDL cholesterol and Apo A-II levels. Serum PON1 activity had a high positive correlation with serum TG levels in

both sexes. Serum ApoB level had a positive correlation with the enzyme activity only in females.

However, Abott CA et al[7] showed that PON1 was only correlated with HDL cholesterol and apoA-1 in the control population in their study.

Bergmeier C et al[41] showed experimentally that the HDL_3 fraction, which is important in reverse cholesterol transport, also carries the highest PON1 activity.

The additional evaluation of the CAD case group by Rozek L et al[42] provided a surprising result, showing strong correlations of PON1 activity with LDL-C and VLDL-C levels and the absence of the expected HDL3 and apoA-I associations. Of further interest, the correlation between PON1 activity and LDL-C and VLDL-C level is positive, whereas low PON1 activity and high LDL-C and VLDL-C levels are predictive of vascular disease.

Sumegova K et al[10] determined PON1 activity, the total antioxidant capacity, as well as entire lipid profile in children aged 11-12 years for screening of possible risk of atherosclerosis development. In children, no significant correlation between PON1 arylesterase activity and HDL was observed. PON1 activity correlated only with atherogenic index. PON1 arylesterase activity was significantly higher in girls than in boys.

MATERIALS AND METHODS

Source of Data

The present study comprised of clinically, diagnostically confirmed cases of nephrotic syndrome, age 2 to 14 years of either sex admitted or attending the pediatric unit of KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum. The study was undertaken over a period of one year from February 2010 to February 2011.

The control group consists of age and sex matched healthy children.

Ethical clearance has been obtained from the Institutional Ethical Clearance Committee, J. N. Medical College, Belgaum.

Method of the Collection of the Data

Sample size calculation: On an average 50 patients of nephrotic syndrome were admitted in the KLE hospital every year in the last consecutive 3 years. 80% of the average admissions per year were taken for the sample size calculation. Hence the sample size for this study was 40 cases

Cases: All cases of nephrotic syndrome in the age group of 2 to 14 years of either sex.

Inclusion criteria

- Clinically, diagnostically confirmed cases of nephrotic syndrome. Following parameters were considered for inclusion of cases in the present study

✓ heavy proteinuria,(>40 mg/m^2/hr in children)

✓ Relapse**:** Urinary protein 3+ or more on 3 consecutive days with or without edema, while in remission.

- ✓ hypoalbuminemia, (serum albumin <2.5gm/dL)
- ✓ hypercholesterolemia (serum total cholesterol>200mg/dL)
- ✓ normal renal function (serum creatinine 0.3-1mg/dL)

Exclusion criteria

- × Familial hyperlipidemia
- × Patients with other renal pathology
- × Patients with liver disease
- × Malnourished children

DATA COLLECTION PROCEDURE

An informed consent was obtained from parents of patients and control group before collecting the blood sample.

Patient details, clinical details and lab results were noted in the proforma.

Collection of blood sample and storage:

5ml of venous blood was collected from the patients and controls under aseptic precautionary measures using disposable syringe in plain tubes. Serum was separated by centrifugation within one hour and analysed within 24 hours or kept at -20^0C if analysis was delayed.

Methods of assay

The following parameters were analysed from the serum sample

1. Total cholesterol – CHOD/PAP Enzymatic method.[43]

2. HDL-C – Polyethylene glycol precipitation method.[44, 45]

3. TG – GPO-PAP enzymatic method.[46]

4. LDL, VLDL – calculated by Friedwald's formula.[47]

Freidewald's formula:

VLDL = Triglycerides/5

LDL = Total cholesterol - (HDL + Triglycerides/5)

Estimation of lipid profile:

Analysed using coral reagent kits from CREST BIOSYSTEMS, INDIA.

Analysed spectrophotometrically in Lawrence and Mayo digital spectrophotometer.

5. Serum protein – Biuret method.[48]

6. Serum albumin- BCG Dye binding method.[48]

7. Serum creatinine – Jaffe's method.[48]

Serum Total protein, albumin and creatinine were analysed using Erba reagents Manual Chemistry kits in Lyopholised form for Semi Auto Analyzers from Erba Diagnostics Manheim gmbh and analysed in Erba- Chem 5 semi autoanalyser.

8.24hr Urinary protein- estimated by Esbach's method using Esbach's albuminometer.[49]

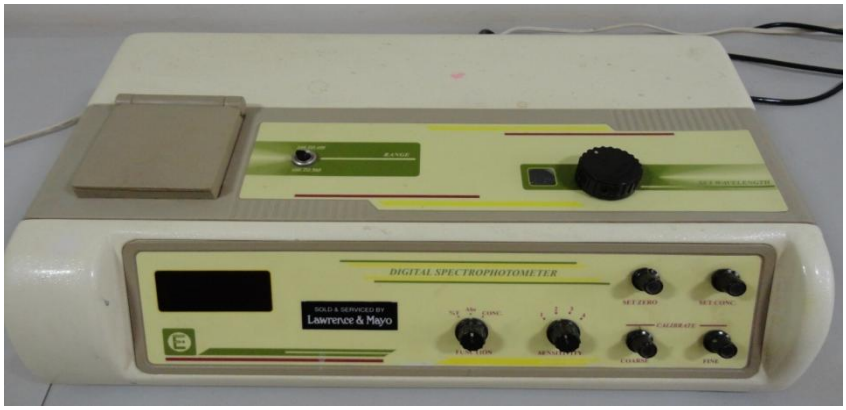


Figure 10: Digital spectrophotometer.

Figure 11:ERBA Chem 5 Semi-auto analyser

Figure 12:Reagent kits and accessories used for various tests.

9.PON1 activity - Spectrophotometric method.[33]

ESTIMATION OF SERUM PON1 BY SPECTROPHOTOMETRIC METHOD[33]

PRINCIPLE:

PON1 activity was determined by using P-nitrophenyl acetate as a substrate. The increase in the absorbance at 402 nm due to formation of P-nitrophenol was measured.

REAGENTS:

1. Solution A

0.25M Tris was prepared by dissolving 30.25 gms of Tris in one liter of double distilled water.

2. Solution B

0.25M HCl was prepared by diluting 21.5 ml of concentrated HCl to one liter double distilled water.

3. Stock Buffer 0.25M of pH 7.4

50 ml of solution A was mixed with 26.8 ml of solution B and the volume was made upto 200 ml and adjusted to pH 7.4.

4. Working Buffer 25 mM/L

Prepared by diluting 1 ml of stock buffer to 10 ml double distilled water.

5. For basal PON1 activity

Tris buffer (25 mM/L) containing 1 mM CaCl2 (11 mg of CaCl2 dissolved in 100 ml of buffer) was prepared.

6. Substrate (5.5mM/L)

15 mg of paranitrophenyl acetate was dissolved in 0.5 ml of absolute ethanol. It was freshly prepared before use.

Enzyme Assay

1. Basal PON1 activity

The activity was measured at 25^0 C by adding 50 µl of serum to 2 ml of buffer containing 1 mM/L CaCl2 in a spectrophotometric cuvette. The initial absorbance was adjusted to 0 with buffer in a spectrophotometer at a wavelength of 402 nm. The reaction was started by adding 50 µl of substrate. The rate of increase in absorbance was monitored over 2minutes at 30, 60, 90,120and 150 seconds.

Non Enzymatic Hydrolysis

2 ml of buffer was taken in spectrophotometric cuvette and 50 µl of substrate was added to it. The rate of change in absorbance **(A)** was monitored at 30, 60, 90,120 and 150 seconds at 402 nm.

The **ΔA** so obtained was deducted from **ΔA** obtained in the presence of serum.

The ΔA (rate of change in absorbance) for 2 min was taken from 30 to 150 seconds for calculating the enzymatic activity. Average of change in absorbance per 30 seconds is calculated and then calculated per minute by multiplying by 2.

The first 30 seconds was not taken for calculation in order to allow the reaction to reach a steady state.

CALCULATION:

Correction for non enzymatic hydrolysis:

Corrected ΔA = Total ΔA – non enzymatic ΔA

PON1 activity was calculated by using molar absorptivity of 14000 M^{-1} cm^{-1} at pH 7.4

Enzyme activity was calculated in U/L by the formula

$$C = \frac{(\Delta A)\ (10^{-6})(TV)}{Є\ (b)\ (SV)}$$

Where ΔA is delta absorbance, difference in absorbance

TV is total volume of sample plus reagents in mL

SV is sample volume used in mL

10^{-6} converts moles to nmol for the IU

Є is the molar absorptivity of p-nitrophenol at 402nm, at 25^0 C and pH 7.4.

b is the light path 1cm.

Substituting the values

$$C = \frac{(\Delta A)\ (10^{-6})(2.1)}{(14{,}000)\ (1)\ (0.05)}$$

$C = (\Delta A) \times 3 \times 10^{-9}$ U/L

STATISTICAL ANALYSIS

The data was analysed by SPSS software. Results are expressed as Mean ± SD. Student's t-test and chi square test was used for group wise comparisons. Relationship between variables was measured by Karl Pearson's correlation co-efficient. A statistical significance is set at 5% level of significance ($p<0.05$).

RESULTS

This study consists of 40 cases of neprotic syndrome in the age group of 2-14 years and 40 healthy controls whose age and sex were comparable to cases. The cases consisted of children in active phase of nephrotic syndrome either newly diagnosed or old cases with relapse.

The distribution of respondents(cases and controls)according to sex and age is given in the **Table 5 and 6**. Out of 40 cases 25 were of male sex while 15 were females. Whereas in controls out of 40, 19 were males and 21 were females. There was no statistical difference in the sex distribution of the two groups (P value 0.178).

Mean age for cases was 6.28 (±3.23) and for controls 7.13 (± 2.45). There was no difference in the mean age between the two groups and the two groups were comparable.

Table 7 shows comparative analysis of TC, TG, HDL, LDL, and VLDL in controls and in cases of nephrotic syndrome. The mean values of TC, TG, HDL, LDL, and VLDL in controls were 168 ± 24.5mg/dL, 129.4 ± 29.6mg/dL, 49.3 ± 6mg/dl, 92.4 ± 24.1mg/dL, 26 ± 5.8mg/dL respectively and the mean values in cases were 364.5 ± 94.6mg/dL, 285.8± 88.2mg/dL, 50.7± 15.5mg/dl, 256.7± 83.3mg/dL, 57.8 ± 18.4mg/dL respectively. Statistical analysis by student's t-test showed that the mean levels of TC, TG, LDL, and VLDL were increased in cases when compared to controls and were statistically significant ($p < 0.001$). The mean levels of HDL were actually slightly higher in cases when compared to controls but not statistically significant.($p=0.610$)

The comparison of various lipid and lipoprotein levels between cases and controls are depicted in the **Graphs 1-5**.

Table 8 shows comparative analysis of PON1 in controls and in cases of nephrotic syndrome. The mean level of PON1 in controls was 302.3±43.4 IU/L and that of cases was 218.7±35.3 IU/L. The levels of PON1 are significantly lowered in cases when compared to controls and is statistically significant.($p<0.001$) The graphical representation of comparison of PON1 levels between cases and controls is shown in **Graph 6**.

It is evident from the **Table 9** that in Cases, there is a positive correlation between TP, ALB, HDL and PON1. i.e, if the level of one parameter increases, the levels of the associated parameter also increases. The correlation between them was not statistically significant ($p>0.05$). It is also observed that there is a negative correlation between TC, TG, LDL, VLDL and PON1 i.e. if the levels of one parameter increase the levels of the associated parameter decreases proportionately. However this was not statistically significant.

In controls TC, TG, LDL and VLDL showed a positive correlation with PON1 and HDL showed a negative correlation. The relation was not statistically significant. ($p>0.05$) The values are given in **Table 10**

Table 5: Distribution of respondents according to gender and study groups

GROUP	MALE	%	FEMALE	%	TOTAL
Cases	25	62.50	15	37.50	40
Control	19	47.50	21	52.50	40
Total	44	110.00	36	90.00	80

$X^2 = 1.818$, DF=1, p=0.178.

Table 6: Mean and SD age of respondents by study groups

Group	Mean age	SD age
Cases	6.28	3.23
Control	7.13	2.45
Total	6.70	2.88

Table 7: Comparison of cases and controls with respect to lipid profiles (TC, TG, HDL, LDL, and VLDL) by t-test

Variable	Group	Mean	SD	t-value	p-value
T.C in mg/dL	Cases	364.5000	94.6705	12.7058	0.0000*
	Control	168.0000	24.5879		
TG in mg/dL	Cases	285.8000	88.2822	10.6153	0.0000*
	Control	129.4750	29.6803		
H.D.L.in mg/dL	Cases	50.7250	15.5398	0.5117	0.6103
	Control	49.3750	6.0751		
L.D.L. in mg/dL	Cases	256.7250	83.3811	11.9719	0.0000*
	Control	92.4250	24.1107		
V.L.D.L. in mg/dL	Cases	57.8250	18.4889	10.3831	0.0000*
	Control	26.0000	5.8266		

*p<0.05

Table 8: Comparison of cases and controls with respect to PON1 (in IU/L) by t-test

Group	Mean	SD	t-value	p-value
Cases	218.7750	35.3448	-9.4295	0.0000*
Control	302.3250	43.4867		

*p<0.05

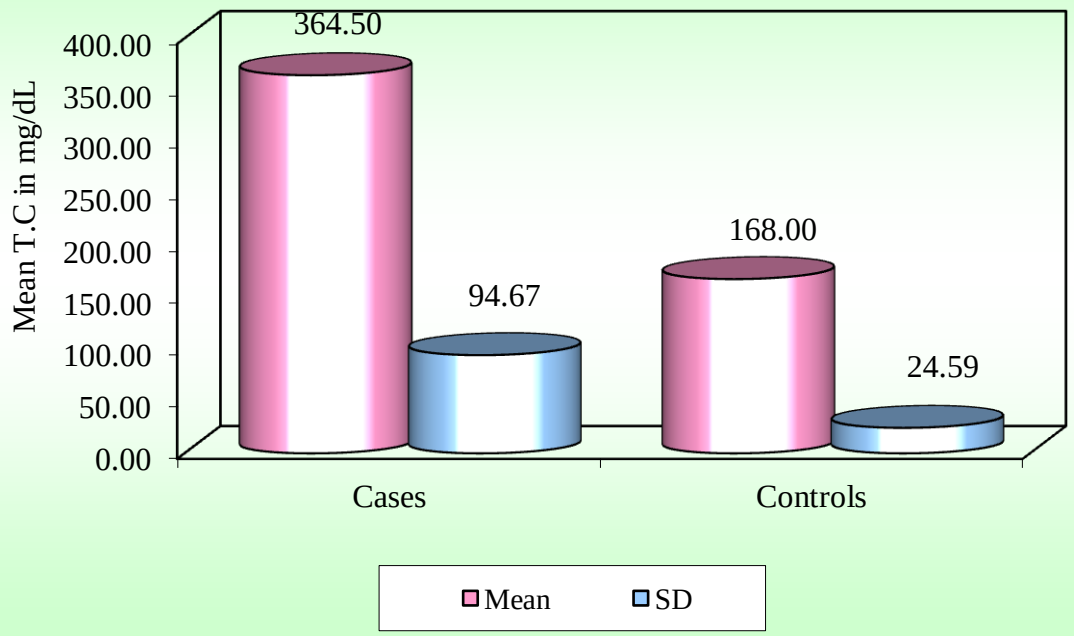
Table 9: Correlation coefficient between T.P.in gm/dL, ALB in gm/dL, , T.C in mg/dL, TG in mg/dL, H.D.L. in mg/dL, L.D.L. in mg/dL and V.L.D.L. in mg/dL with PON1 by Karl Pearson's correlation coefficient method in cases

Parameters	Correlation coefficient between PON1 with		
	Correlation coefficient	t-value	P-value
T.P.in gm/dL	0.0855	0.5292	0.5997
ALB in gm/dL	0.0726	0.4490	0.6560
T.C in mg/dL	-0.2246	-1.4210	0.1635
TG in mg/dL	-0.2498	-1.5903	0.1200
H.D.L. in mg/dL	0.1030	0.6381	0.5272
L.D.L. in mg/dL	-0.2141	-1.3513	0.1846
V.L.D.L. in mg/dL	-0.2613	-1.6685	0.1034

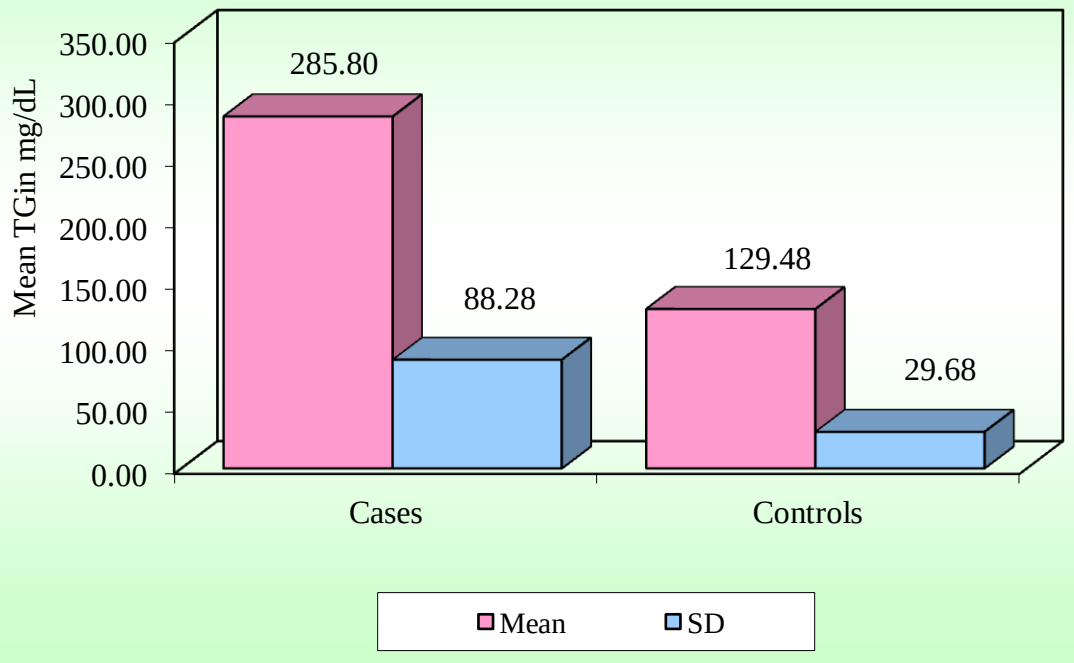
Table 10: Correlation coefficient between T.C in mg/dL, TG in mg/dL, H.D.L. in mg/dL, L.D.L. in mg/dL and V.L.D.L. in mg/dL with PON1 by Karl Pearson's correlation coefficient method in controls

Parameters	Correlation coefficient between PON1 with		
	Correlation coefficient	t-value	P-value
T.C in mg/dL	0.0799	0.4938	0.6243
TG in mg/dL	0.2463	1.5665	0.1255
H.D.L. in mg/dL	-0.0850	-0.5259	0.6020
L.D.L. in mg/dL	0.0581	0.3589	0.7217
V.L.D.L. in mg/dL	0.2550	1.6258	0.1123

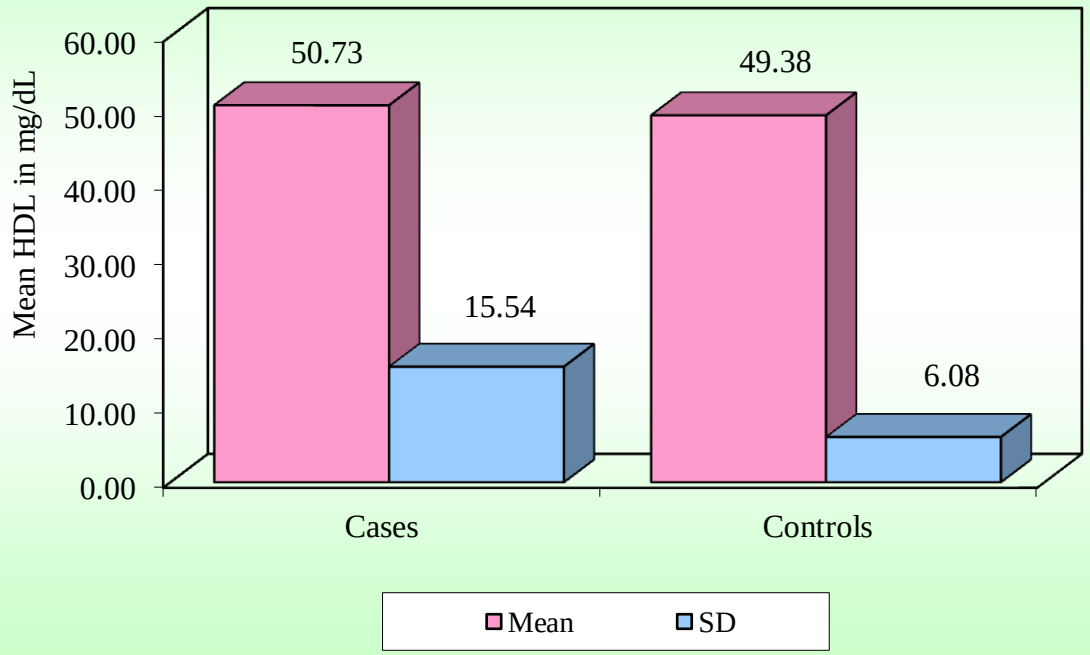
Graph 1: Comparison of cases and controls with respect to lipid profile i.e.T.C in mg/dL



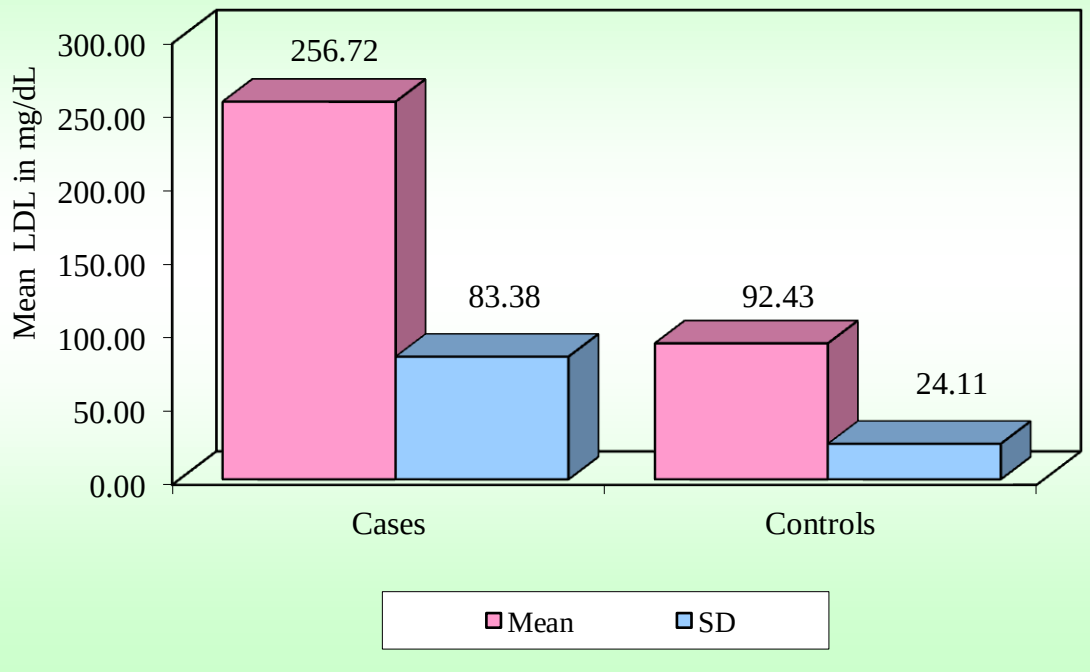
Graph 2: Comparison of cases and controls with respect to lipid profile i.e.TG in mg/dL



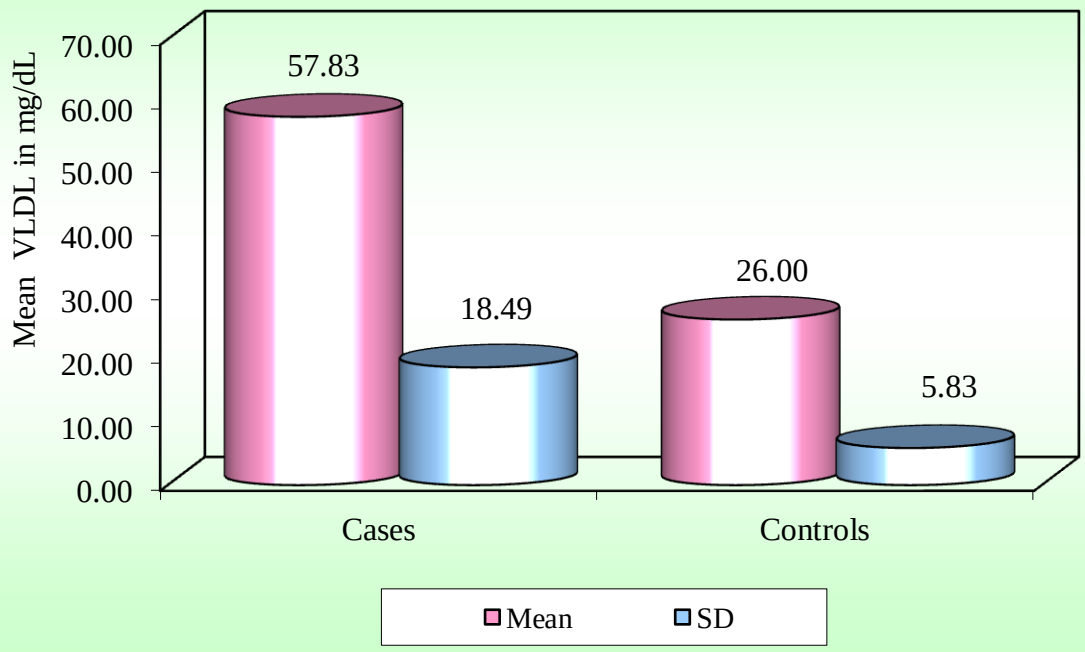
Graph 3: Comparison of cases and controls with respect to lipid profile i.e.HDL in mg/dL



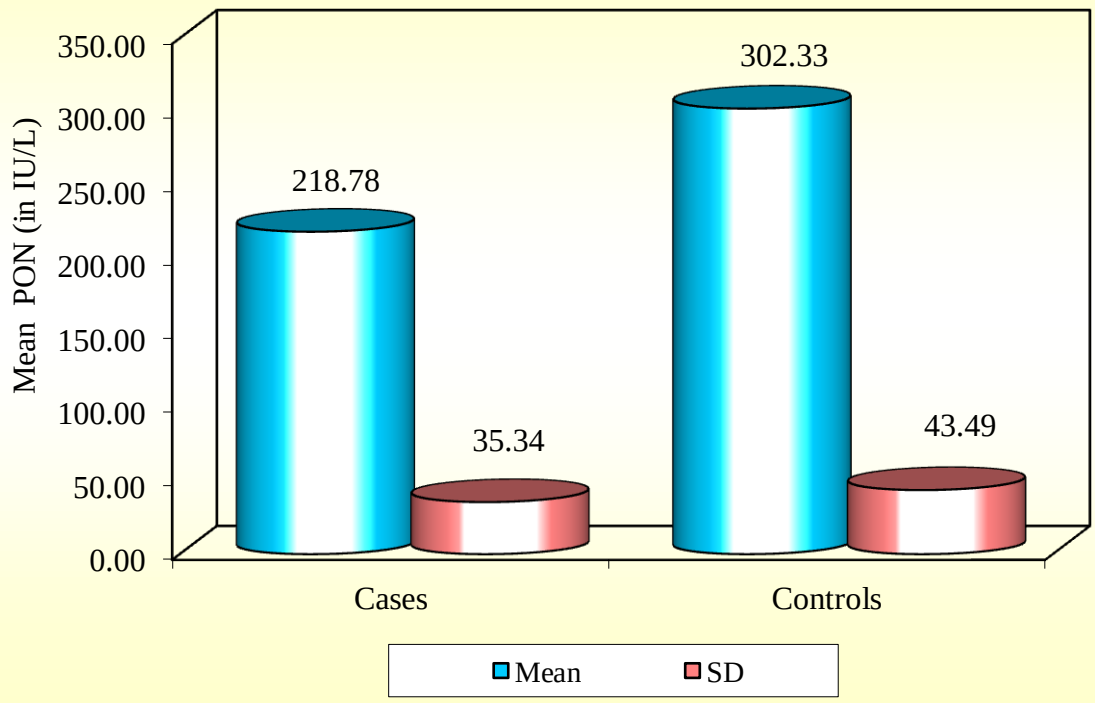
Graph 4: Comparison of cases and controls with respect to lipid profile i.e.LDL in mg/dL



Graph 5: Comparison of cases and controls with respect to lipid profile i.e.VLDL in mg/dL



Graph 6: Comparison of cases and controls with respect to PON1 (in IU/L)



DISCUSSION

We measured the serum lipid concentrations and PON1 activity in 40 cases of nephrotic syndrome in the age group of 2-14 years. Children in active phase of nephrotic syndrome either newly diagnosed or old cases with relapse and 40 healthy controls whose age and sex were comparable to cases were taken. In agreement with previous studies [3,15,38,50,51] our patients had increased mean levels of TC, TG, LDL, and VLDL in comparison to controls and were statistically significant ($p < 0.001$). In contrast to the study done by El-Melegy et al[3] in 2008, who found lower levels of HDL in cases, we found that the mean levels of HDL were actually slightly higher in cases when compared to controls but within normal range. This is in accordance to study done by Muls et al[52] who found normal levels of HDL in NS and also reported abnormal distribution of HDL subtypes. Also Hu P et al[51] observed higher levels of HDL in cases compared to controls. There is increased levels of HDL_3 and decreased HDL_2[13,16,52] which measured together give normal values for total HDL but result in defective reverse cholesterol transport pathway**.**

Hyperlipidemia and dyslipidemia in nephrotic syndrome occurs due to abnormalities in lipoprotein metabolism i.e. increased synthesis of VLDL, LDL, decreased catabolism of VLDL and LDL and altered reverse cholesterol transport pathways .The exact cause for these disturbances is not yet clear. Proteinuria, and not an increased rate of albumin synthesis, plays a causal role in nephrotic hyperlipidemia.[13,21] These results suggest that urinary loss of a substance that regulates lipid metabolism may play a key role in pathogenesis of NS. Turnover studies using radiolabelled glycerol and mevalonate have shown an increase in synthesis of TG and cholesterol. Increased lipoprotein production could be linked to

increased supply of substrate. Since albumin is the acceptor of free fatty acids a decreased albumin to free fatty acid ratio in NS could make more free fatty acid available for synthesis of lipoprotein.[13] Impaired renal mevalonate metabolism may lead to increased mevalonate concentrations and thus raised hepatic production of cholesterol.[12,16] HMG CoA reductase activity is also increased in hepatocytes. This will lead to enhanced VLDL production and decreased expression of LDL receptors, thereby reducing the rate of cholesterol clearance from the circulation. Finally increased hepatic production of proteins, for example CETP mediates transfer of esterified cholesterol from HDL to TG rich lipoproteins VLDL. In some nephrotic patients synthesis of LDL apo B100 was actually greater than VLDL apo B100 suggesting an alternate pathway bypassing the normal delipidation pathway for increased LDL synthesis.[22]

According to Kaysen et al[22], while VLDL synthesis may be increased in some NS cases, VLDL levels increase predominantly because of decreased VLDL clearance. Measurement of LPL activity have demonstrated impaired enzyme function, this being a major cause of catabolic defect. Enzymes may be simply lost in the urine or alternatively, since albumin augments LPL by binding free fatty acids, a product of lipoprotein hydrolysis, hypoalbuminemia may result in free fatty acid accumulation which inhibits enzyme activity.[12,16] But analbuminemia did not affect this activity. Thus urinary loss of a LPL co factor may contribute to reduced activity.[16] Candidate molecules that may influence LPL activity and can be lost in urine in NS are (i) apo CII, increased CIII/CII ratio.[14,16] (ii) HDL- impaired reverse cholesterol transport.[23] (iii) loss of glycosaminoglycans i.e heparin sulphate which anchors LPL to endothelium.[12,16] (iv) increased FFA:albumin ratio.[13]

Reduced activity of another key enzyme in lipoprotein catabolism LCAT has also been documented.[12,16] Low LCAT impairs cholesterol esterification within the HDL particle thereby inhibiting conversion of HDL_3 to HDL_2. This inturn reduces transfer of apo CII to VLDL and thus inhibits catabolism of TG rich lipoproteins.[24] Another abnormality that may contribute to nephrotic hyperlipidemia is defective removal of IDL and LDL from circulation via lipoprotein receptors as their expression is decreased.[16] There is increased lipid uptake by scavenger receptors in activated lymphocytes or macrophages which is unregulated.

All these measures contribute to dyslipidemia of NS. In addition, the study of Ece et al[39] observed that steroid treatment did not suppress oxidative stress or corrected lipid abnormalities in SSNS. The results of study done by researchers suggest that nephrotic children may have prolonged periods of hyperlipidemia even after clinical remission.[53] Moreover, frequently relapsing children were more likely to have abnormal lipid profile during the remission.[54] Lipid abnormalities seen in nephrotic syndrome constitutes atherogenic dyslipidemia and can predispose to subclinical atherosclerosis in adulthood, which has practical implications for coronary artery disease risk assessment and intervention in pediatric populations.[55]

Further we also found in this study, the levels of enzyme PON1 that hydrolyses oxidized lipids, is decreased significantly in cases when compared to controls. This is in accordance to studies done by researchers Ece et al[39] and Kniażewska M H.et al[6,] Studies have reported that cases of nephrotic syndrome have increased oxidative stress and decreased antioxidant defense system. Also there is evidence for endothelial dysfunction[56], increased lipid oxidation and impaired activity of some antioxidant enzymes[57] in the acute phase of INS that, along with

hyperlipidemia, may increase the risk of early atherosclerosis development.[3, 6, 39, 58] PON1 activity was decreased not only in active phase but also in remission while on steroids.[39] Our results show highly increased levels of lipoproteins. As a result of increased oxidative species, levels of oxidized LDL are increased. The oxidative modification of LDL represents an important pathway in the pathogenesis of atherogenesis. Studies have also reported significantly elevated levels of ox LDL in cases of nephrotic syndrome.[3] Pathology studies have established that atherosclerotic disease begins in childhood and will progress faster if risk factors are present.[59] With hyperlipidemia in NS, there is increased accumulation of lipoproteins in intima of blood vessels which due to changes induced by free radicals get oxidized to ox LDL and ingested by macrophages via scavenger receptors forming foam cells. Once ox LDL saturates macrophages and smooth muscle cells start accumulating lipids, the process of atherosclerosis becomes progressive and cannot be reversed back.[59] The antioxidants present within both LDL and the microenvironments in which LDL is trapped function to prevent the formation of these biologically active, oxidized lipids. Enzymes associated with LDL and HDL (eg, platelet activating factor acetylhydrolase) or with HDL alone (eg, PON1) destroy these biologically active lipids.[26,57] The results of another study have established a negative correlation between HDL-PON1 activity and the levels of lipid hydroperoxides associated with HDL and LDL which confirm the relationship between PON1 activity and lipid peroxidation of lipoproteins.[60] Hence reduced PON1 in these cases predispose them to atheroscleorsis by preventing by hydrolysis of ox LDL.

In agreement with Aydın Ece et al[39], we found a positive correlation between TP, ALBUMIN and PON1 in cases. We also found a positive correlation between HDL and PON1 in cases, and negative correlation in controls. This is in contrast to

the studies done by the same researchers who found no correlation between them.[39]Not many studies are done in this topic but studies in adult control subjects have shown a positive correlation.[7, 61,62] We found a negative correlation between TC, TG, LDL, VLDL and PON1 in cases, whereas in controls there was positive correlation . However our correlation studies were not statistically significant. This is in contrast to a study done in healthy children in Slovakia who found no correlation between PON1 and lipid parameters.[10] In a study in adult population there was a positive correlation between PON1 and LDL, VLDL in cases with vascular diseases and in controls PON1 correlated with HDL.[42] That the relationship of PON1 and lipid profile is different in NS cases versus controls in our study suggests other factors influenced PON1 activity more in cases than in controls. One of these factors may be the co-regulation of the PON1 activity with lipoprotein levels. Since the correlation was different and opposite in cases and controls, it is difficult to explain the regulation of PON 1 activity. In conclusion, PON1 activity contributed to the prediction of the genetic architecture of lipid, lipoprotein, levels differentially in NS cases and controls. This suggests that the joint regulation of PON1 with lipoproteins differs in these two groups, offering one explanation for the consistent finding that PON1 activity predicts risk of atherosclerosis.

Albumin is the major circulating antioxidant in plasma. Decreased albumin levels in active phase of NS may also be related to alterations in antioxidant status of nephrotic patients. Serum PON1 is another component which is known to retard the oxidation of LDL by preventing the generation of lipid peroxides. The antioxidant activity of HDL cholesterol is due largely to PON1

Reduced PON1 activity could also be a result of increased oxidative stress and/or decreased HDL levels. In our study we found normal levels of HDL and

reduced levels of PON1.[39]This could be explained by the abnormal distribution of HDL subtypes in NS. The HDL_2 is decreased and HDL_3 fraction is elevated in NS which also carries the highest PON1 activity[41] and is lost in urine in nephrotic syndrome. Further low serum proteins, such as insufficient antioxidants, may lead to greater consumption of PON1.[39] These antioxidant and anti-inflammatory properties of HDL may be as important as its cholesterol efflux function in terms of protecting against the development of atherosclerosis. Mechanisms against oxidative stress are very important in protection and tapering down the rate of progression in nephrotic syndrome.[26]

In total except for HDL cholesterol all lipid and lipoproteins, including serum cholesterol and LDL cholesterol were significantly higher in cases than controls. Also PON1 activity is significantly decreased in these cases. Though correlation between lipid profile and PON1 activity in our cases was found, it was opposite to association seen in controls and did not reach statistical significance. All these measures collectively constitute an important link between nephrotic syndrome and atherosclerosis. The pathophysiology of nephrotic dyslipoproteinemia is multifactorial. Hence there is a rationale for treatment with lipid lowering drugs like the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase–inhibiting drugs to treat the hyperlipidemia seen in nephrotic syndrome. Also reports state improvement in PON1 activity during treatment with statins.[27,63] However, the benefits of treatment with lipid-lowering drugs have not been proven and should be evaluated.

CONCLUSION

This study clearly shows a significant increase in TC, TG, LDL and VLDL with normal HDL together with reduced PON1. All this will lead to increased oxLDL formation during the disease period contributing to atherosclerotic process in children with NS. Also the correlation of PON1 with lipid profile is different in cases and controls suggesting different mechanisms regulating its activity in both cases.

As atherosclerotic process begins in childhood and is accelerated if risk factors are present and the life expectancy of children and adolescents affected with NS has dramatically improved over the last 15 years, the occurrence of dyslipidemia with its associated morbidity is of particular concern. Because of the paucity of interpretable long term clinical data and the difficulty in performing prospective studies attention is given to the pattern of hyperlipidemia in assessing risk of atherosclerosis. These children are at an increased risk and we suggest that these cases must be followed up to assess the development of atherosclerosis and measures should be taken to lower the lipids and normalize the PON1 activity to prevent complications.

LIMITATIONS OF THE STUDY

1) Both newly diagnosed and relapse cases were included in the study.
2) HDL subtypes were not estimated separately.
3) Ox LDL which would be a better marker to estimate the risk of atherosclerosis and correlate with PON1 activity was not measured due to cost reasons.

SUMMARY

We took up this study with the aim of estimating the lipid and lipoprotein levels and PON1 activity in cases of NS and also to find if there exists any correlation between them.

The present cross sectional study was conducted at Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum between February 2010 to February 2011 on 40 cases of idiopathic nephrotic syndrome and 40 healthy controls in the age of group of 2 to 14 years of both sexes. Lipid profile i.e, TC, TG, LDL, VLDL, HDL were estimated in all the cases and controls, so was PON1. The age and sex of the cases and controls were comparable. There was significant elevation in TC, TG, LDL and VLDL in cases compared to controls. HDL levels were in the normal range in both groups. PON1 activity was significantly decreased in cases than controls. The findings of this study suggest a atherogenic dyslipidemic profile in cases of NS and reduced capacity to prevent oxidation of lipids as evidenced by reduced PON1 activity. Together this provides an important link between NS and atherosclerosis.

We found a positive correlation between HDL and PON1 in cases and a negative correlation between TC, TG, LDL, VLDL and PON1. In controls, the correlation was opposite. However the correlation study was statistically insignificant and cannot be explained. This may imply that the regulation of PON1 and lipid metabolism may be different in cases and controls.

Therefore cases of NS are at increased risk of atherosclerosis and measures should be taken to lower lipid levels and improve PON1 activity to prevent them from premature atherosclerosis.

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ANNEXURE I - CONSENT FORM

INFORMED CONSENT (MODEL OF THE CONSENT FORM IS ENCLOSED) DESCRIBING THE FOLLOWING

Mr./Mrs/Ms. ……

you are invited to participate in our research study that is a study to know the Paraoxonase activity and lipid profile in nephrotic syndrome. A cross sectional study

Participation in this study is completely voluntary. All the patients with nephrotic syndrome and equal number of healthy children will be enrolled in this study at J. N. Medical College, Belgaum under the supervision of Dr. Anuradha B. Patil $_{MD}$, Associate Professor, Department of Biochemistry, J. N. Mediwcal College, Belgaum. The study will be carried out by Dr. Vijayetha S.Kagwad, P.G. Department of Biochemistry, K.L.E. University, Belgaum for her M.D. dissertation to be submitted to KLE University, Belgaum.

PURPOSE OF THE STUDY

Nephrotic syndrome is one of the common chronic disorders causing dyslipidemia and hence it is essential to identify the candidates at risk at an early stage by estimation of PON and lipid profile so that they can be put on diet therapy or pharmacological therapy to prevent premature atherosclerosis.

PROCEDURE

For both the groups that is those with nephrotic syndrome (cases) and healthy subjects (control) 5ml of venous blood will be collected under aseptic precautionary measures using sterile disposable syringe..

RISKS

Since the blood is drawn under aseptic precautionary measures by trained persons there is no scope for any risks. Further only small volume of blood is collected which will be spontaneously replenished in the body. However there may be minor risks associated with having blood drawn that may include bruising, redness, discomfort or bleeding at the puncture site.

BENEFITS

No direct benefit is guaranteed to you from participating in our study. You can make use of blood levels of studied parameters if desired.

OPTIONS

If you decide not to participate in this study, the hospital will provide you the usual standard care and treatment.

NEW INFORMATION

Does not apply to this research.

PRIVACY AND CONFIDENTIALITY

All information collected about you during the course of the study will be kept confidential to the extent permitted by law. You will be identified in this research record by the code numbers. Information which identifies you personally will not be revealed without your written permission. However your records may be revealed to the sponsor of the study. Information from this study may be published but your identity will be confidential in any publication.

INSTITUTIONAL POLICY

In the event that you are physically injured as a result of participating in this research emergency care will be available. There is no commitment to provide any compensation for research related injury. The J. N. Medical College will provide, within the limitations of the laws of the state of Karnataka, facilities and medical attention to subjects who suffered any harm as the result of your participation in this study. In the event you believe that you have suffered any how as a result of your participation in this study you may contact research guide Dr. Anuradha B. Patil $_{MD}$, Associate Professor, Department of Biochemistry.

COST FOR PARTICIPATION

You will not be charged for the test to be carried out on your blood sample.

FINANCIAL INCENTIVE FOR PARTICIPATION

You will not receive any remuneration for participating in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

If you decide not to participate in this study, it will not affect the quality of the medical care you receive at this institution.

You may withdraw from the study anytime. The researchers might use the information learned from the study in scientific journal articles or in presentations.

In case you have any questions regarding your rights as a study participant, you may please contact Dr. V. D. Patil, Principal, J. N. M. C., KLE University, Belgaum and Chairman of J. N. M. C. Institutional Ethics Committee of Human Subjects Research, Telephone No. 0831-2471701.

EMERGENCY PROVISION

If you have questions as a participant in our study, you can contact the study investigator Dr. Vijayetha S. Kagwad, Mobile No. 9449015978 or the research guide Dr. Anuradha B. Patil $_{MD}$, Associate Professor, Department of Biochemistry, Phone No. 0831-2473777 (Extension) 1522.Mobile no.9481738133.

CONSENT TO PARTICIPATE IN A RESEARCH TRIAL

I voluntarily agree to take part in this study. If I choose to take part in the study, I may withdraw at anytime. I am not giving any of my legal right by signing this form. My signature below indicates that I have read, or had read to me, this entire consent form including the risks and benefits. I may ask questions at any time.

_____ _____

Signature of participant Date

_____

Participants Name (Printed):

_____ _____

Name and Signature of witness-1 Date

_____ _____

Name and Signature of witness-2 Date

_____ _____

Signature of researchers or Person obtaining consent

Date

ANNEXURE II : PROFORMA

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

Paraoxonase activity and lipid profile in nephrotic syndrome. A cross sectional study

Name : Sex :

Age : IP No :

DOA :

Address:

Presenting Complaints: Chief complaints-Generalized Oedema

Oliguria

Hematuria

Dysuria

Fever

H/o Secondary causes of Nephrotic Syndrome : Skin rash and joint pains , Drug History , Jaundice and blood Transfusion

Past history

History of similar episode in the past.

History of treatment with steroids for that episode.

No. and frequency of relapses.

Family history

Similar complaints in any of family members.

Family History of kidney diseases: Yes/No

Personal history

Sleep

Appetite

Bowel and bladder habits

General Physical Examination

PR:……./min R.R.: ……/min

BP.: ……mm Hg Pallor Oedema

Anthropometry: Ht

Wt

HC

Nutritional Status: Normal

PEM

Systemic Examination:

P/A

CVS

RS

CNS

Investigations

Hb : TC : DC : ESR :

Serum Protein

Albumin

Urea

Creatinine

Urine: Albumin Pus Cells Casts

USG Abdomen

Special Investigations

Blood :

Total cholesterol

HDL

Triglyceride

LDL, VLDL

Paraoxonase

Urine: 24 hr Urine protein

Final diagnosis

ANNEXURE – III: MASTER CHART

Cases Group

Sl.no	Name	Age in yrs	Sex	T.P.in gm/dL	ALB in gm/dL	CRE in mg/dL	T.C in mg/dL	TG in mg/dL	H.D.L. in mg/dL	L.D.L. in mg/dL	V.L.D.L. in mg/dL	PON1 in IU/L
1	L	4	F	4.1	2.5	0.5	369	412	55	232	82	219
2	U	5	M	4.5	2.4	0.8	323	259	52	249	51	237
3	SI	11	M	4.6	2.4	0.8	446	365	55	318	73	219
4	RU	7	F	3.8	2	1	352	419	56	212	83	207
5	SA	6	F	3.9	1.4	0.4	488	381	44	368	76	228
6	RI	10	F	4	1.7	0.6	400	295	56	285	59	240
7	MAL	9	M	4.6	1.5	0.7	496	410	47	367	82	204
8	PR	7	F	4.9	2.1	0.6	360	238	39	273	47	210
9	SH	5	F	3.8	2	0.8	320	333	47	206	66	178
10	AK	9	F	5	2.5	0.8	272	119	36	212	23	273
11	SIM	4	F	4	1.9	0.5	214	181	69	108	36	240
12	NI	9	F	4.2	1.8	0.5	214	281	58	99	56	252
13	MA	6	M	4.4	2.2	0.6	260	190	50	172	38	225
14	RA	10	M	4.6	2.1	0.6	322	276	78	188	55	246
15	MANI	5	F	4.5	2.4	0.9	501	333	100	334	66	240
16	PRA	4	M	4	1	0.9	260	217	38	181	43	216
17	Y	12	M	4.1	1.6	0.9	488	382	41	371	76	225
18	AY	2	M	4.2	1.2	0.5	488	382	41	371	76	210
19	DE	12	M	3.5	1.4	0.9	357	203	47	274	41	205
20	AF	3	M	3.3	1.5	0.5	600	320	93	443	64	181
21	JU	6	M	3.9	1.7	0.8	479	304	39	379	61	206
22	TA	2	M	3.5	1.4	0.6	418	384	48	295	77	219
23	BA	12	M	4.1	2.5	0.9	501	378	43	379	77	180
24	PRT	2	M	4.8	2.6	0.3	256	113	43	190	23	238
25	AB	4	M	5	2.9	0.5	253	127	37	191	25	217
26	RA	4	M	5.2	2.6	0.7	385	262	70	263	52	208
27	SAH	8	F	4.9	2.1	0.8	301	210	39	218	44	185
28	TA	9	F	3.5	1.2	0.6	408	305	36	317	61	199
29	MAH	5	M	4.8	2.4	0.7	310	294	48	207	55	220
30	SU	4	M	3.9	1.5	0.6	348	290	38	252	58	258
31	DA	3	M	6	2.1	0.7	240	190	53	149	38	247
32	BH	2	F	3.5	1.5	0.5	464	240	44	372	48	280
33	DK	14	M	3.8	1.6	1	400	240	44	308	48	117
34	RAK	5	F	3.6	1.3	0.5	382	440	47	247	88	280
35	KI	3	M	4.8	1.9	0.5	333	425	47	201	85	105
36	BJ	4	M	4	1.7	0.5	320	256	30	242	48	218
37	PPA	6	M	5	2.6	0.6	224	181	64	119	39	240
38	MDA	4	M	4.2	2.2	0.5	315	225	32	238	45	228
39	SH	9	F	4.1	2	0.6	391	296	47	251	93	205
40	SHD	5	M	4.6	2.1	0.5	322	276	78	188	55	246

Control Group

Sl.no.	Name	Age in yrs	Sex	T.C in mg/dL	TG in mg/dL	H.D.L. in mg/dL	L.D.L. in mg/dL	V.L.D.L. in mg/dL	PON1 in IU/L
1	AN	10	M	173	102	56	97	20	231
2	LA	10	F	181	137	42	112	27	261
3	ADA	12	F	135	133	42	66	27	324
4	ATA	10	F	153	135	42	84	27	300
5	AK	10	M	129	115	42	64	23	216
6	APA	13	F	140	123	48	67	25	345
7	Y	9	M	133	131	42	65	26	244
8	SYA	9	F	169	170	48	79	34	216
9	ZA	9	F	157	103	46	90	21	330
10	YU	8	M	143	206	45	57	41	372
11	SNA	8	F	157	133	48	82	27	324
12	KA	8	F	160	138	46	86	28	252
13	MSA	7	F	154	135	40	87	27	330
14	SFA	8	F	143	120	48	71	24	288
15	MDS	8	M	155	200	38	77	40	411
16	JU	8	F	193	126	40	128	25	315
17	TA	7	F	179	160	45	102	32	324
18	GE	7	F	175	120	48	103	24	285
19	ABV	7	M	204	126	49	130	25	280
20	PRI	6	F	174	143	52	93	29	300
21	RKA	10	F	242	209	54	146	42	298
22	MMT	3	F	171	143	58	84	29	315
23	RYA	4	F	154	160	52	70	32	340
24	VK	4	M	165	131	56	83	26	288
25	CHI	4	F	180	129	46	108	26	324
26	CTN	5	M	247	130	44	177	26	330
27	DYA	8	F	207	126	52	130	25	390
28	FA	5	F	160	106	58	81	21	280
29	KN	5	M	160	138	58	75	27	290
30	MHT	5	M	165	144	56	80	29	268
31	JO	4	M	172	126	52	95	25	312
32	PO	5	F	171	98	54	97	20	285
33	SMN	4	M	170	89	54	98	18	268
34	AKY	5	M	172	78	58	98	16	350
35	SUB	5	M	151	110	58	71	22	362
36	AL	4	M	175	94	52	103	20	310
37	BHT	6	M	155	106	56	78	21	320
38	NE	7	M	155	82	48	89	18	285
39	ABH	8	M	174	110	44	108	22	272
40	AKH	10	M	167	114	58	86	23	258

KEY TO MASTER CHART

Sl. No.	-	Serial Number
Yrs	-	years
F	-	Female
M	-	Male
gm	-	gram
mg	-	miligram
dL	-	deciliter
IU	-	international units
L	-	litre
T.P	-	Total protein
ALB	-	Albumin
CRE	-	Creatinine
T.C.	-	Total cholesterol
TG	-	Triglycerides
H.D.L.	-	High density lipoprotein cholesterol
L.D.L.	-	Low density lipoprotein cholesterol
V.L.D.L.	-	Very low density lipoprotein cholesterol
PON1	-	Paraoxonase